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21 FEBRUARY 1967

ASSEMBLY/STERILIZER FACILITY FEASIBILITY PROGRAM FINAL REPORT

VOLUME I

CONTRACT NO. NAS 1-5381

GENERAL ELECTRIC
RE-ENTRY SYSTEMS DEPARTMENT

THIS FINAL REPORT CONSISTS OF TWO VOLUMES:

- VOLUME NO. I -- CONTAINS SECTIONS I THROUGH IV
- VOLUME NO. II - CONTAINS APPENDICES A THROUGH E

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VOLUME I

CONTRACT NO. NAS 1-5381

PREPARED FOR

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I. INTRODUCTION

Programs for interplanetary missions such as Voyager, require sterile flight vehicles if they will enter the atmosphere or land on the surface of extra-terrestrial planets. Two basic concepts have been proposed for the achievement of the sterility of the spacecraft. These are called "Terminal Sterilization" and "Assembly/Sterilizer" processing.

Terminal sterilization refers to the concept in which a vehicle is assembled and checked out in a clean room, decontaminated with a gaseous biocidal agent (ETO), sealed in a cannister, and then exposed to dry heat sterilization. Subsequent to sterilization no access to the flight vehicle is possible without recontamination.

The Assembly/Sterilizer concept was developed by GE and was first presented to NASA in the Mariner B and Voyager system design studies in 1963. The Assembly/Sterilizer is an ultra-bioclean-room facility which allows decontamination and sterilization of a disassembled spacecraft, and subsequent assembly, checkout, adjustment, and if necessary, repair of the spacecraft in a sterile environment. All human operators are topologically and biologically isolated from the spacecraft after sterilization operations have been initiated.

A study program to investigate the feasibility of the Assembly/Sterilizer concept was started by GE on 21 July 1965 under contract to the Langley Research Center of the National Aeronautics and Space Administration. The contract was completed on 21 February 1967.

The results of the investigation indicate that the Assembly/Sterilizer concept is feasible.

A. Program Objectives

The objective was to provide the initial demonstration of the Technical feasibility of a facility which would permit the decontamination and sterilization of spacecraft. Envisioned also, was the further capability for subsequent checkout, adjustment, repair, and encapsulation of the payload in a biological barrier under sterile conditions. An analog of an Assembly/Sterilizer facility was prepared which could accomplish these objectives without the necessity of erecting a large facility. Concurrent with this program, a design study was initiated to incorporate the results of the tests in progress into a facility plan which would reflect the knowledge acquired during this program.

B. Program Ground Rules

The program as prescribed in Appendix A was divided into four tasks which, it was felt, would give the widest possible scope to the study. They were:

1. Test Sample
2. Test Program and Demonstration
3. Bio-assay
4. Full Scale Facility Design Study

An effort was made to ensure that the test sample used in this program would be representative of prime hardware commonly used throughout the aerospace industry. The sample, during assembly, was of sufficient complexity that its assembly created some difficulties for the operator chosen to work on it. The parts making up the assembled component, after withstanding decontamination and sterilization procedure, were required to function flawlessly.

Little was known concerning the physical limitations of personnel who would be required to work in a facility of this type. Further knowledge was desirable also from a standpoint of personnel reactions to specific problems introduced at various increments in the program.

It also became necessary, early in the program, to investigate the ability of available tools to withstand sterilization requirements. The tools selected were those necessary for completion of the task assigned and not of every type and variety available. The test program as initiated was designed to assure that the analog after installation was achieving the required decontamination and sterilization objectives. This portion of the program was divided into 3 parts.

- Sterilization Verification
- Manipulation
- Feasibility Demonstration

The first part was necessary to verify that the chambers were achieving the required goals using the prescribed treatments. The second part involved the performance of assigned tasks under progressively more restrictive conditions, and the third part consists of simulating actual assembly under various conditions, thereby assuring the capability of the analog.

To carry out the above assignments, and in so-doing meet the necessary sterilization criteria, it was necessary to perform biological assays on samples located in the analog. Since component parts and normal atmosphere contain a variable amount of viable organisms, a portion of the test program consisted of introducing known numbers of viable organisms into the chambers and, after prescribed treatments, conducting a bio-assay to determine the survival rate. A large quantity of sterility control specimens were prepared in the laboratory and used during the test program.

The final task, as envisioned, was to use the information as gathered to assist in the design stages of the full scale facility study. A number of plans were considered throughout the test program, but after due deliberation and comparison, the number under consideration was reduced and a more detailed study ensued. The plan selected is described in detail in Appendix E.

C. Summary of Conclusions

The collected data encompassing all the tasks set forth in this program, and described in greater detail herein, demonstrated the feasibility of the Assembly/Sterilizer concept. Present-day aerospace hardware can be assembled under "clean room" conditions, decontaminated, sterilized, and still perform its designed function with no loss of response. Human operators, if carefully

selected, can perform fairly complex assembly operations under such conditions with little discomfort. The tools used in the program were limited only by their ability to withstand the sterilization cycles. Tools such as screwdrivers with plastic handles, and those containing moving parts, were found incompatible with sterilization techniques.

All of the objectives set forth in Appendix A relative to the bio-assay portion were successfully met. Large microbial populations were reduced significantly using Ethylene oxide, between three to eight decades in most cases.

All the cycles performed in the analog were successful, inthat no living organism could be detected as having survived after assembly, insertion and/or repair subsequent to sterilization.

II. PROGRAM ACTIVITY AND ACCOMPLISHMENTS

A. TASK 1. TEST SAMPLE

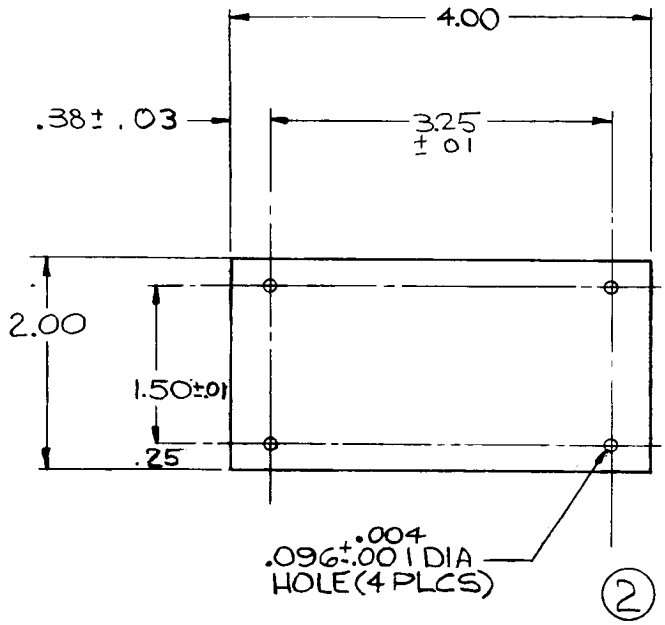
1) DESIGN

In performance of the studies of this contract, it was necessary to perform demonstration tests consisting of typical sterilization, assembly, checkout, and packaging operations in the Assembly/Sterilizer Analog and to perform biological assays to demonstrate the achievement and maintenance of sterility. To create the greatest realism in these demonstration tests and the most accurate analog of Full-Scale Assembly/Sterilizer operations, the use of actual spacecraft hardware would have been desirable. However, the high unit cost and the number of components required for a meaningful demonstration would have resulted in excessive test hardware costs. Furthermore, since qualified dry heat sterilizable components are not in the present inventory of spacecraft hardware, the design and qualification of components would have represented prohibitive and unjustified expenditure of time and funding.

A second approach would have been to sterilize and assemble groups of "parts" of simple geometric shapes made of stainless steel. Using this sterile "tinkertoy" approach would have permitted a simplified biological sampling situation; and if conic forms were used, the test specimens could have been good visual analogs of spacecraft. However, the resulting specimens would not have been any more than visually analogous to any realistic spacecraft equipment, and would have had no useful performance capability.

A compromise was made between the use of qualified spacecraft equipment and the "tinkertoy" blocks. By designing a simplified test sample component it was possible to achieve most of the benefit of either of the above without being restricted by high cost on the one hand or lack of realism on the other. It was considered necessary that the optimum components should employ a reasonable range of spacecraft materials, electronic parts, and assembly techniques, and should be capable of a useful performance function. The test sample selected was a printed circuit stage mounted on supporting brackets which are in turn attached to an element of spacecraft skin bonded to heat shield material.

The test sample, Figure II-1, was fabricated of an aluminum skin, two inches by four inches by forty thousandths thick to which was bonded a one quarter inch thick ESM heat shield which contained a thermocouple. Mounted on this basic structure, by means of brackets and supports, was an epoxy glass printed circuit board containing the electronics circuitry which included transistors, resistors, diodes, and capacitors. The circuit of the test sample, Figure II-2, in concurrence with Langley technical representatives, was

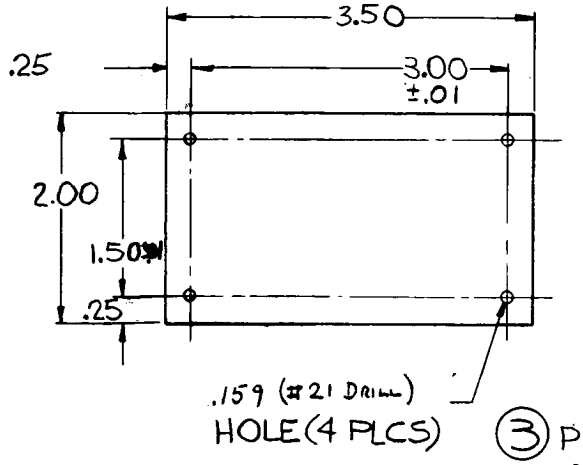


NAS1068C
(4 REQ'D)
NUT PLATES

TACK WELD THESE
2 EDGES PRIOR T
ASS'Y (2 PLCS)

.096 +.0 -.0
HOLE(2

② SKIN
.040 THK
ALUM-QQ-A-250
6061-T6



③ PRINTED CIRCUIT BR
.064 THK EPOXY GLAS

NOTES:

1. ALL TOLERANCES ±.03 UNLESS OTHERWISE SPECIFIED.
2. INSTALL RIVETS PER 118A1508.
3. OPEN END-POP RIVETS: UNITED SHOE MACH. CORP. AD 32 BS SHELDRON. CONN. 06485
4. ADHESIVE RTV560* WITH 0.5% T-12 CATALYST (CLEAN & PRIME WITH GE4004 BEFORE APPLYING ADHESIVE.)
5. ESM 1004AP SERIES NOMINAL THICKNESS 1/4 IN.
6. HOLE FOR IRON CONSTAN THERMOCOUPLE TO BE HELD IN PLACE BY RTV 560* OR RTV60

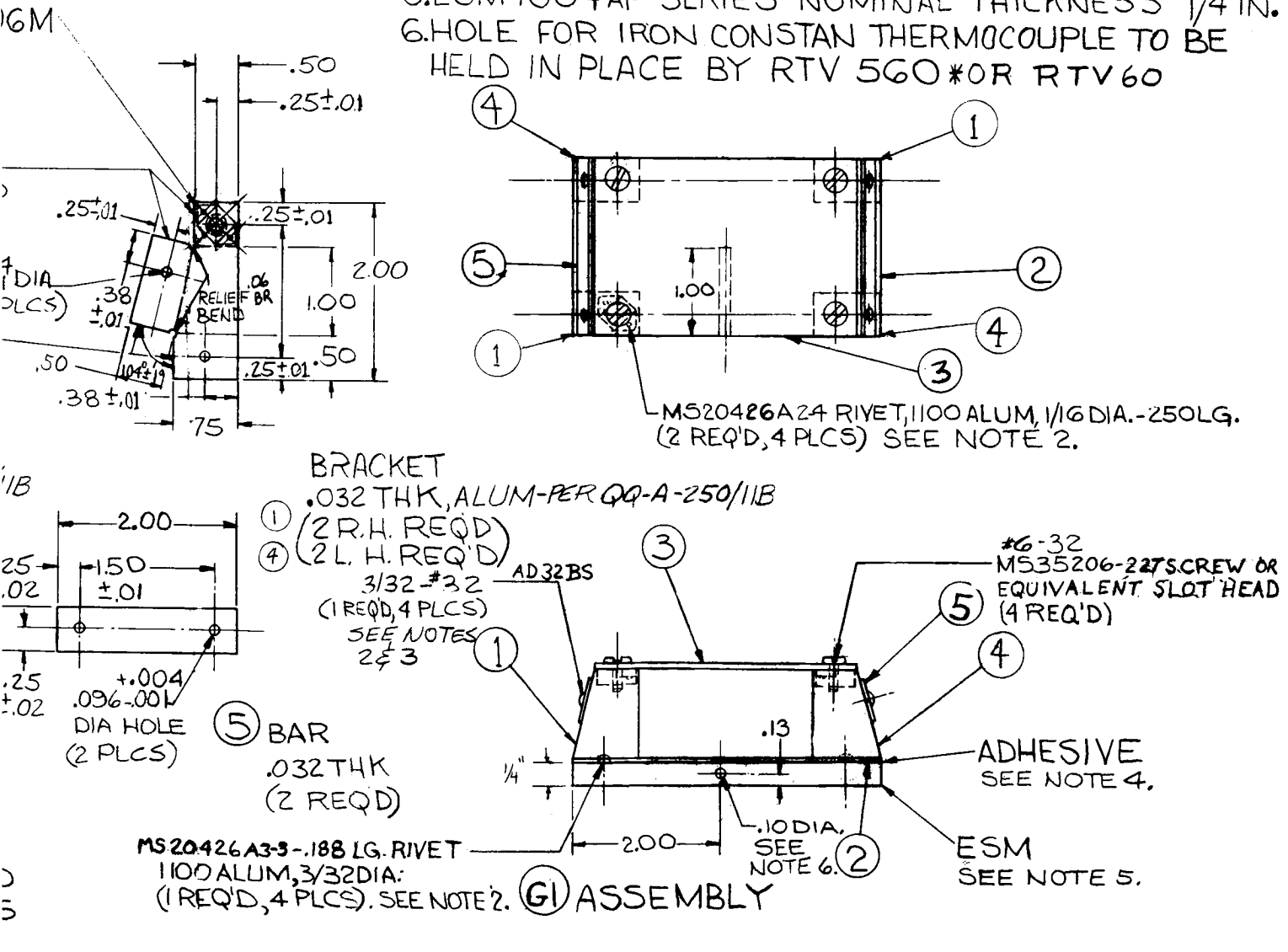
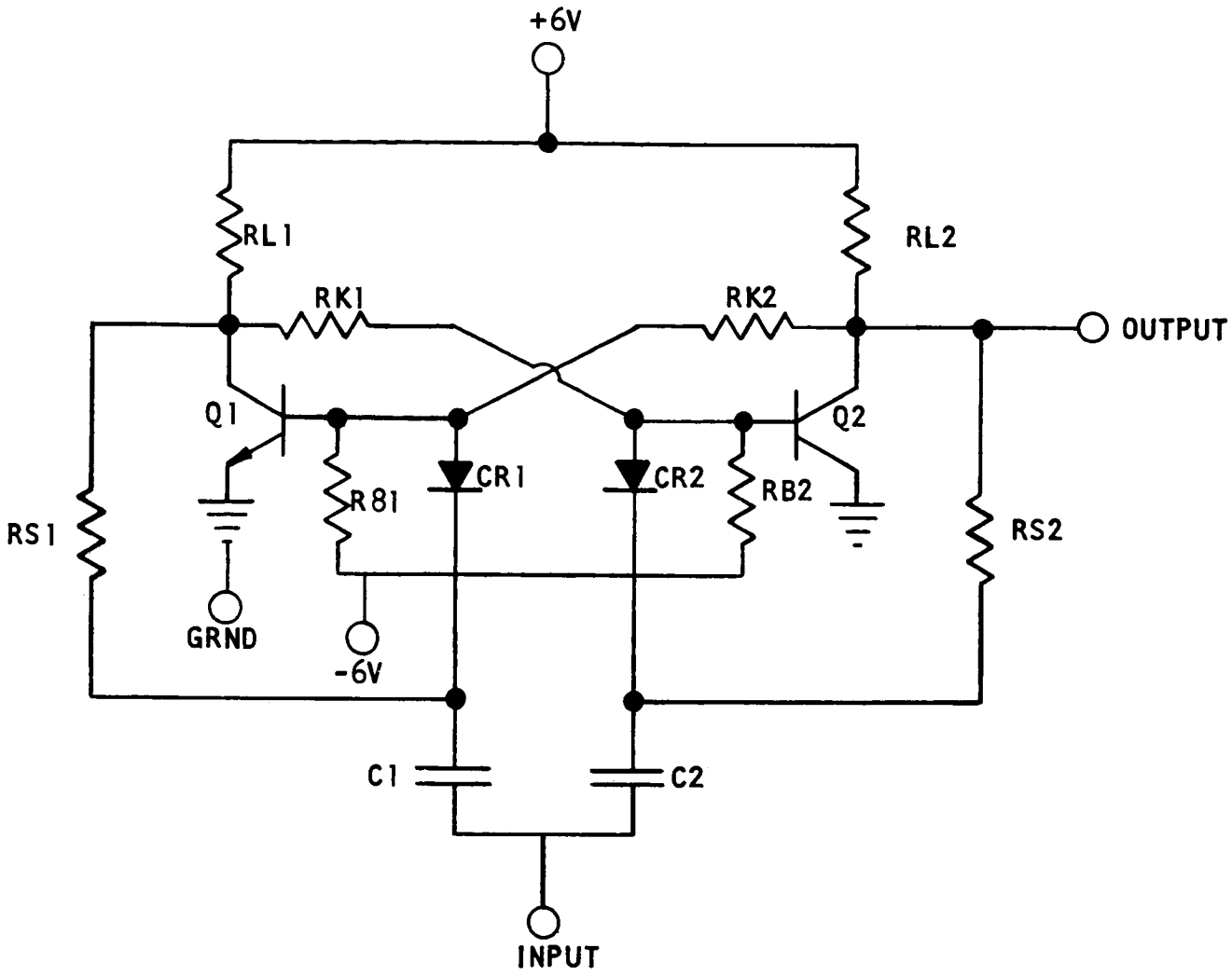


Figure II-1 - Test Sample For Assembly/Sterilizer(SK-56117-802)

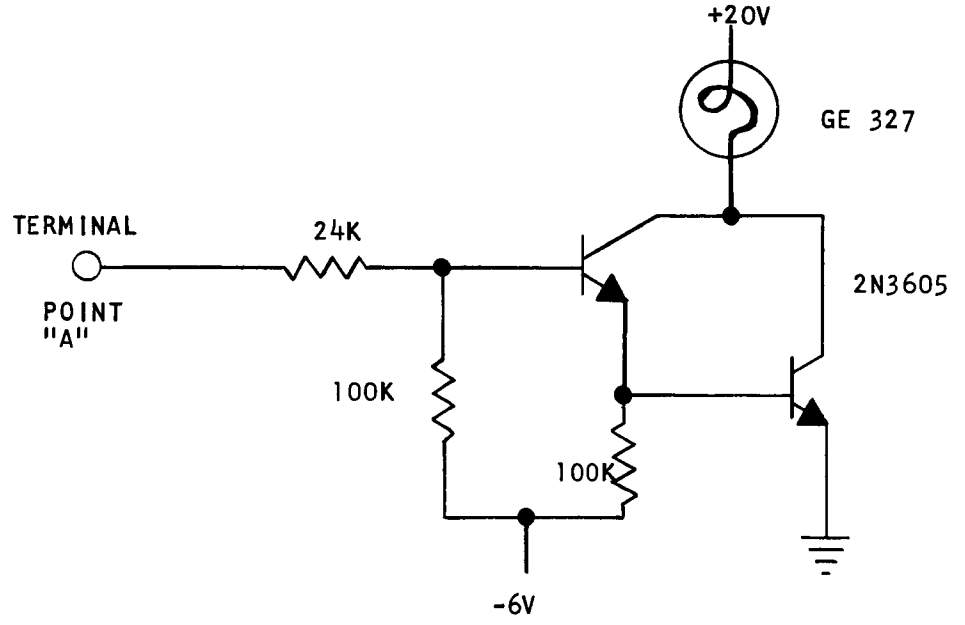


RL1,RL2 -- 1K, ± 5% RC07
RK1,RK2 -- 3.9K, ±5% RC07
RB1, RB2-- 100K, ±5% RC07
RS1, RS2-- 10K, ±5% RC07
C1, C2 -- 470 pF, CK05CW471K
Q1, Q2 -- 2N706
CR1,CR2 -- 1N914
5 Terminals R2574P2

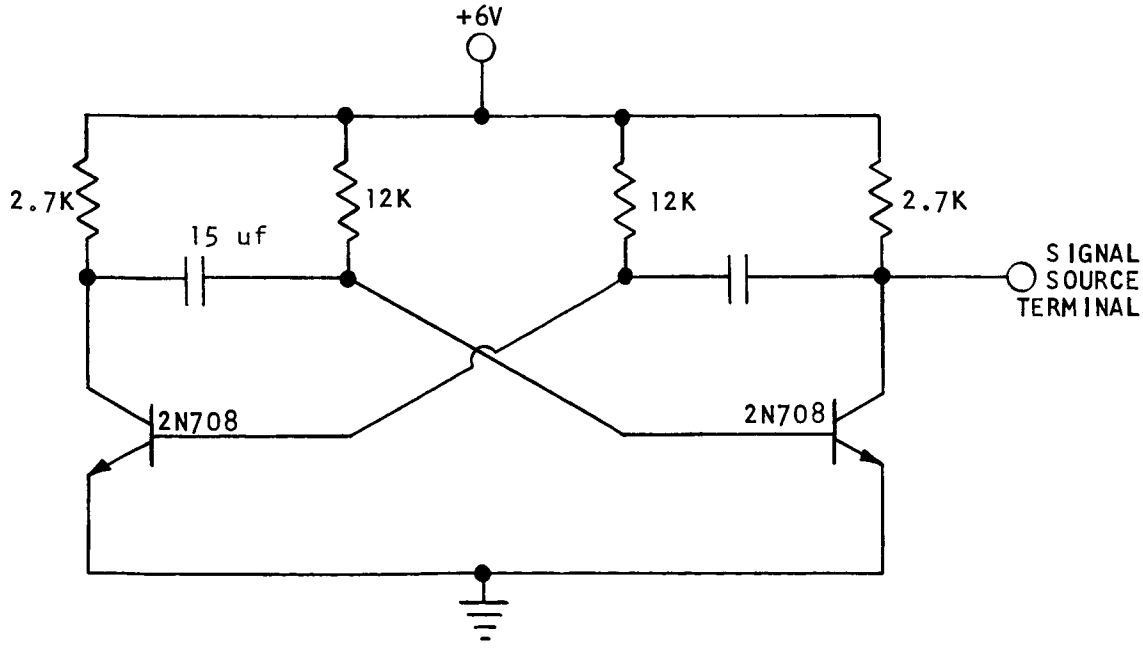
Figure II-2-Test Sample Circuit

modified by changing from a one-stage audio amplifier, as originally proposed, to a triggered multivibrator. This was done in order to present a more typical hardware-type representative of an unmanned planetary lander.

A test set was required to drive the binary counter of the test sample and to monitor its response. The test set as illustrated in Figure II-3 consisted of a signal generator and an output indicator.



a. Indicator



b. Signal Generator

Figure II-3 Test Sample Test Set

2) MANUFACTURING

The manufacturing flow diagram of Figure II-4 shows the orderly flow of materials from receipt of raw materials to final assembly.

Since a large percentage of the assembly of a prime interplanetary spacecraft will be done under class 100 clean room conditions, it was deemed desireable to assemble the test sample printed circuit boards under class 100 conditions. Therefore, a class 100 laminar flow bench was employed as the work location for assembling the test sample printed circuit boards. To determine the bio-environment in the laminar flow bench, Reynier slit air samplers and stainless steel fall-out strips were used to monitor the populations of organisms flowing across the assembly area in the bench, and the organism fallout within the bench. To provide an indication of the bio-environment improvement provided by the bench, air samples were taken in the controlled environment area in which the laminar flow bench was located. The results of the bench and room assays are reported under Task 3.

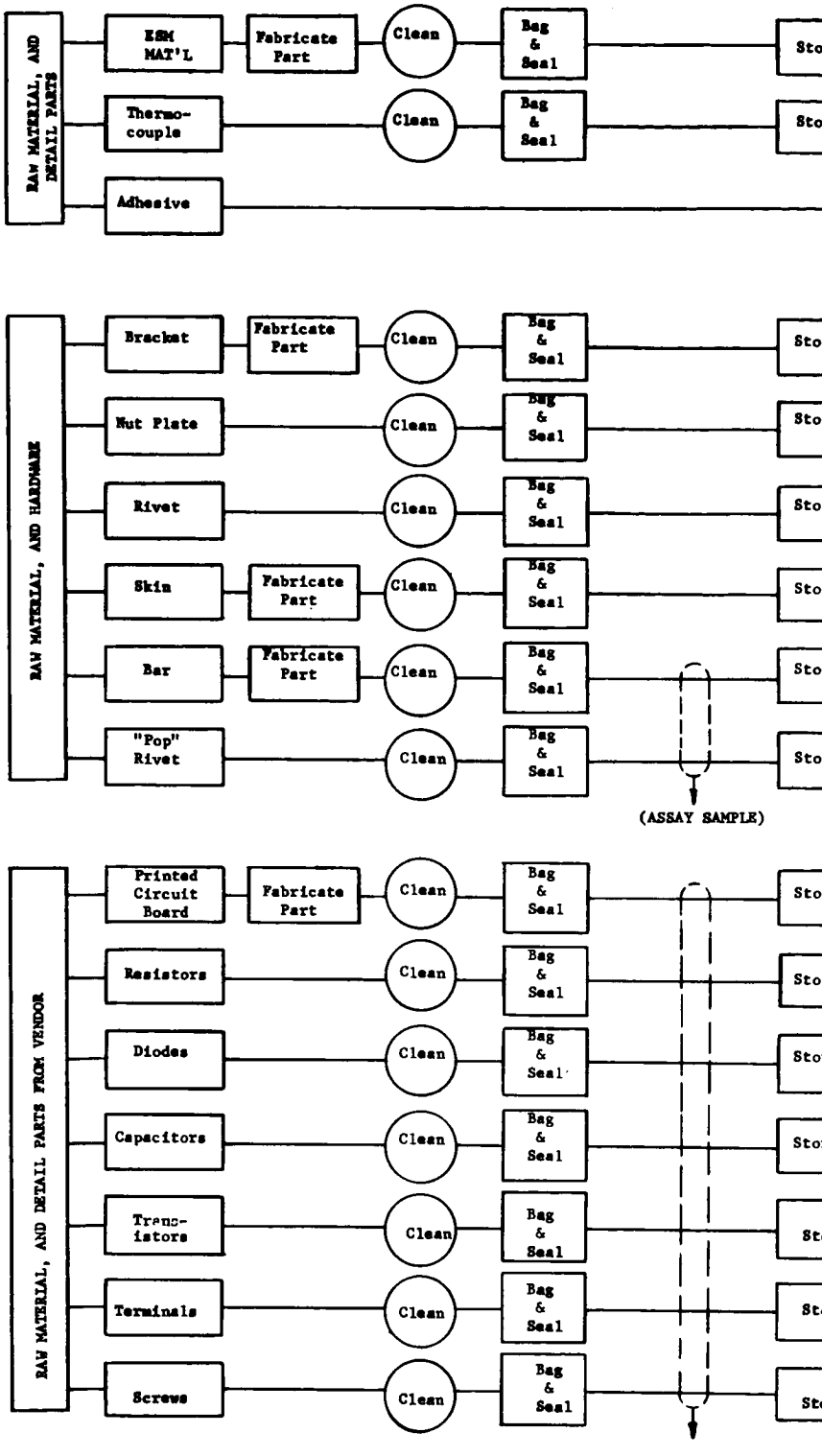
Figures II-5, through II-7 show the clean bench assembly set-up. Figure II-5 shows the full bench top with tools, parts, printed circuit holding fixture, Reynier slit air samplers, and four trays of fall-out strips.

Figure II-6 shows a closer view of the working area of the bench. The fixture is holding a partially wired test sample printed circuit board. To the right of the fixture are the technicians tools. Behind the fixture are two fall-out strip trays. To the left of the fixture are three plastic bags. The one closest to the fixture contains a completed board. Next to the completed board is a bag containing an unwired board, and next to that are the bagged parts for this board.

Figure II-7 shows the technician working on the board. He wears a lint free smock and cap, a surgical mask, and finger cots. The bench and technician are located in a controlled environment area which is an ante-chamber to the GE horizontal laminar flow clean room at 3198 Chestnut Street.

Figure II-8 below, shows two views of a manufacturing prototype of the test sample. This prototype was used to confirm metal working tooling and shows the mechanical configuration of the test sample.

Figures II-9A and 9B are illustrations of the final "Prime test sample. This sample is one of those used in the demonstration cycles of Task 4.


RAW MATERIAL, AND DETAIL PARTS
ESM MAT'L
Fabricate Part
Clean
Bag & Seal
Sto
Thermo-couple
Clean
Bag & Seal
Sto
Adhesive
RAW MATERIAL, AND HARDWARE
Bracket
Fabricate Part
Clean
Bag & Seal
Sto
Nut Plate
Clean
Bag & Seal
Sto
Rivet
Clean
Bag & Seal
Sto
Skin
Fabricate Part
Clean
Bag & Seal
Sto
Bar
Fabricate Part
Clean
Bag & Seal
Sto
"Pop" Rivet
Clean
Bag & Seal
Sto
(ASSAY SAMPLE)
RAW MATERIAL, AND DETAIL PARTS FROM VENDOR
Printed Circuit Board
Fabricate Part
Clean
Bag & Seal
Sto
Resistors
Clean
Bag & Seal
Sto
Diodes
Clean
Bag & Seal
Sto
Capacitors
Clean
Bag & Seal
Sto
Trans-istors
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Bag & Seal
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Terminals
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Bag & Seal
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Screws
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Bag & Seal
St

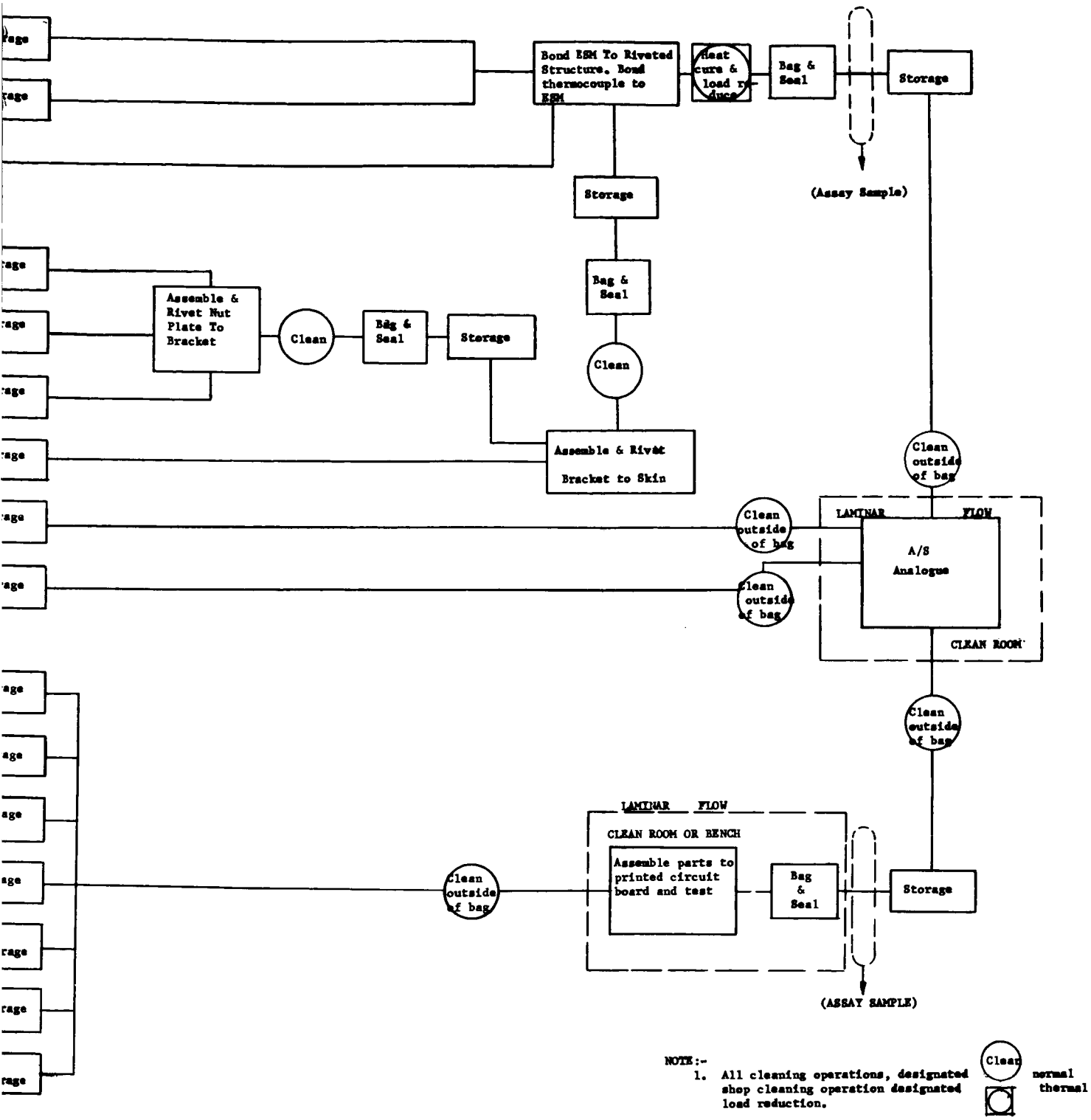


Figure II-4 - Assembly Flow of Test Sample

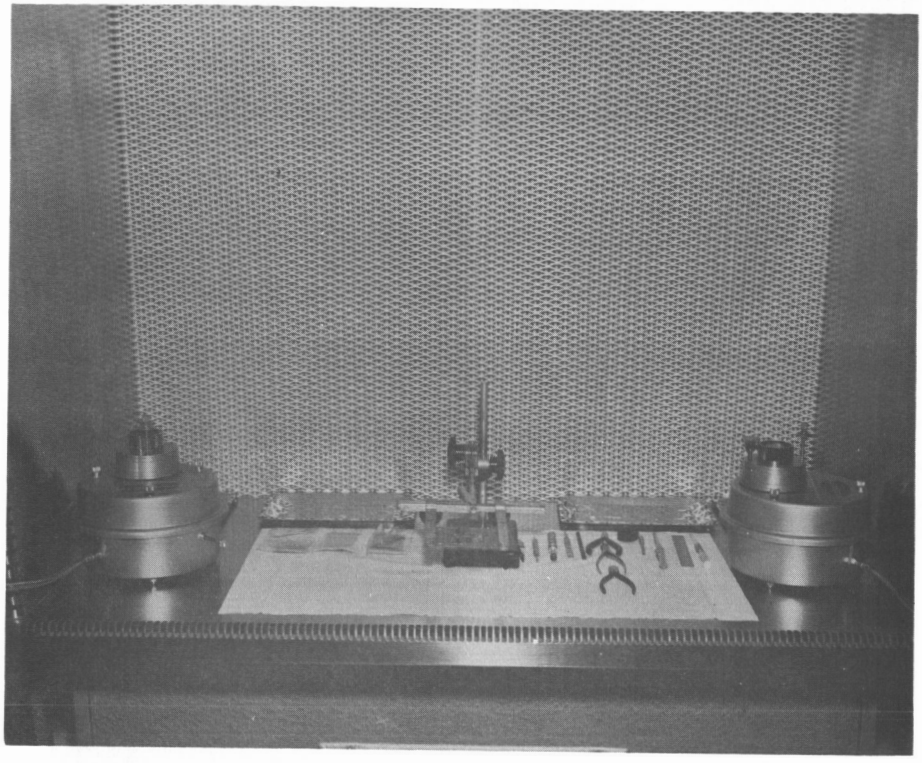


Figure II-5 Class 100 Clean Bench Set-Up

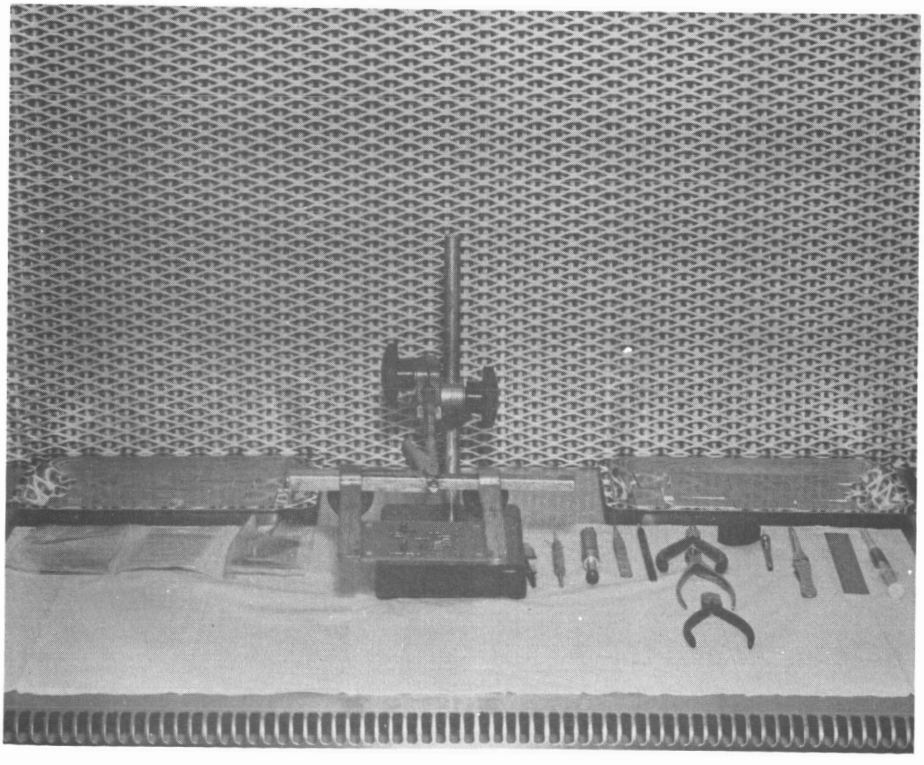


Figure II-6 Class 100 Clean Bench Close-up

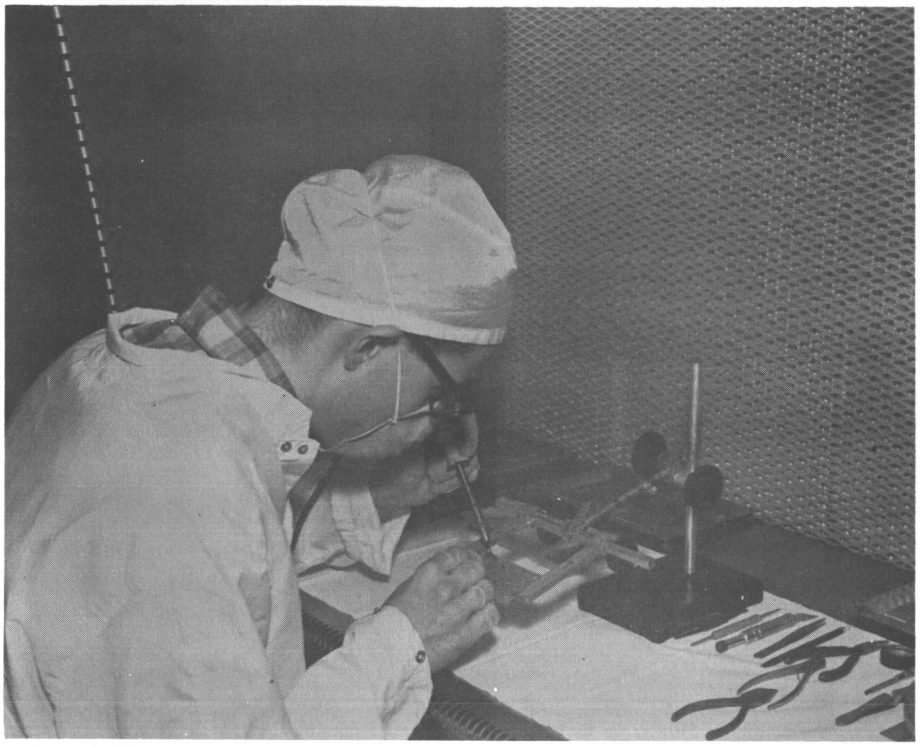
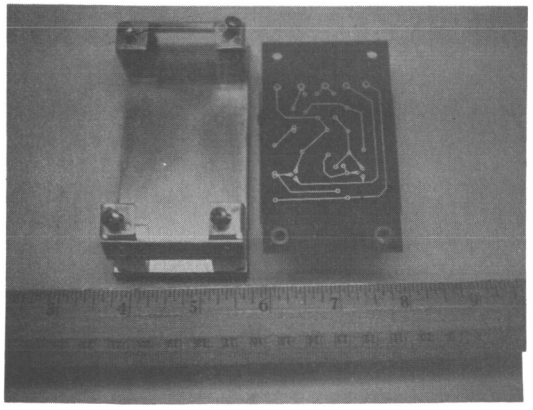


Figure II-7 Assembly of Test Sample Printed Circuit Board



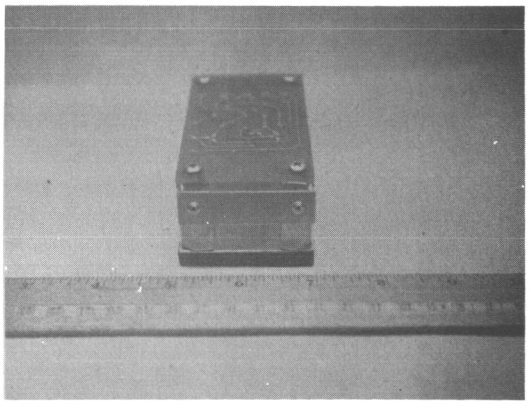


Figure II-8 Test Sample Manufacturing Prototype

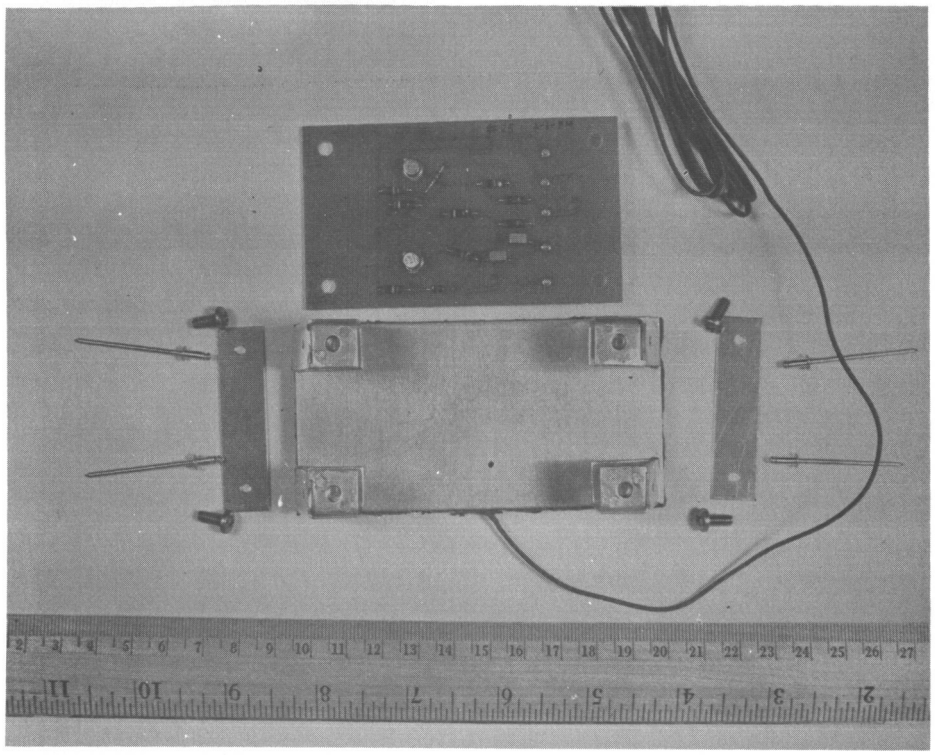


Figure 9A - Disassembled "Prime" Test Sample

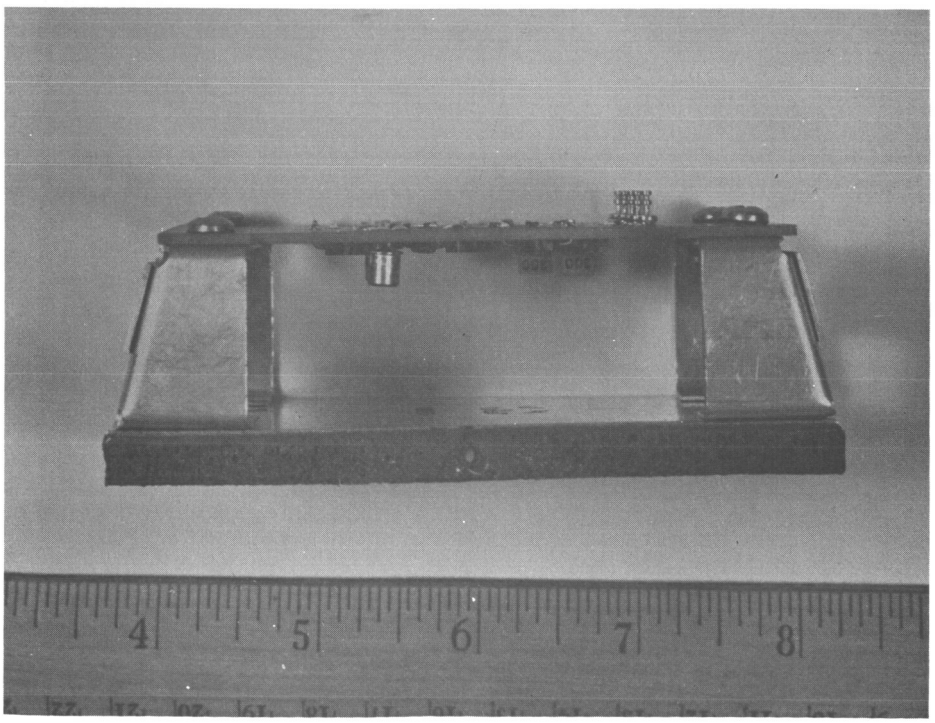


Figure 9B - Assembled "Prime" Test Sample

3) TESTING

To establish the suitability of the test sample for the program, a breadboard of the circuitry was exposed to one cycle of sterilization, 24 hours at 135 degrees C., followed by a 20 hour operational life test. The procedure for this test was to perform the following steps.

1. Operate the test sample breakboard and photograph input and output waveforms on a dual trace oscilloscope.

2. Soak test circuit for 24 hours at + 135°C (non-operating)

3. Repeat step #1.

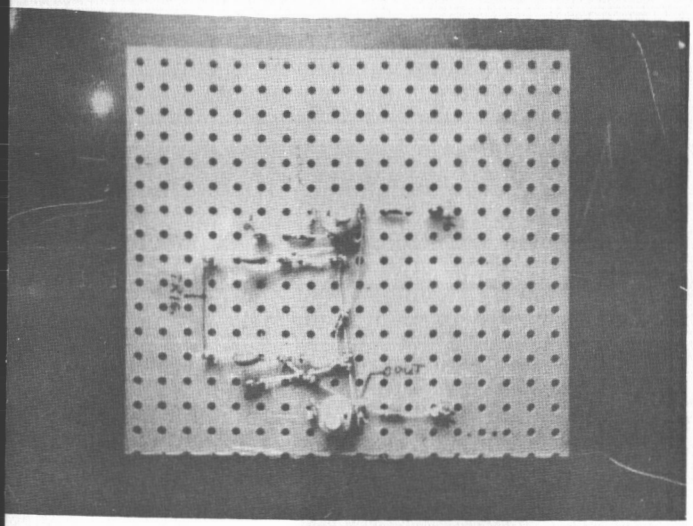
4. Operate test circuit for 20 hours (accumulated hours of operation) repeating step #1 every two hours.

Monitored oven temperature during the temperature soak period of the test ranged from 272 to 276°F.

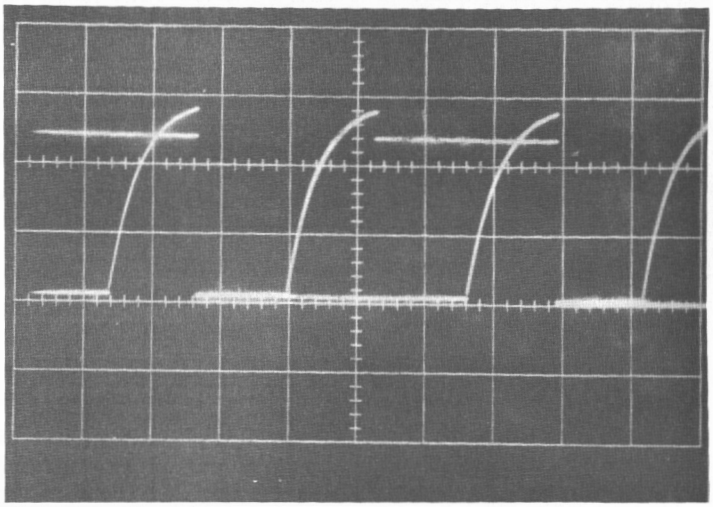
Table II-I and Figure II-10 show the breadboard and the input and output waveforms recorded during the test. Both input (sawtooth wave) and output (square wave) signals were fed into a Tektronix plug-in amplifier Model CA operated in the chopped mode. The waveforms were superimposed upon each other for display on the oscilloscope screen. The amplifier of the dual trace oscilloscope used to monitor the input and output waveforms was checked and calibrated with the Internal calibration of two similar oscilloscopes. The test sample test set provided the drawing signal and test load for the test sample.

TABLE II-I

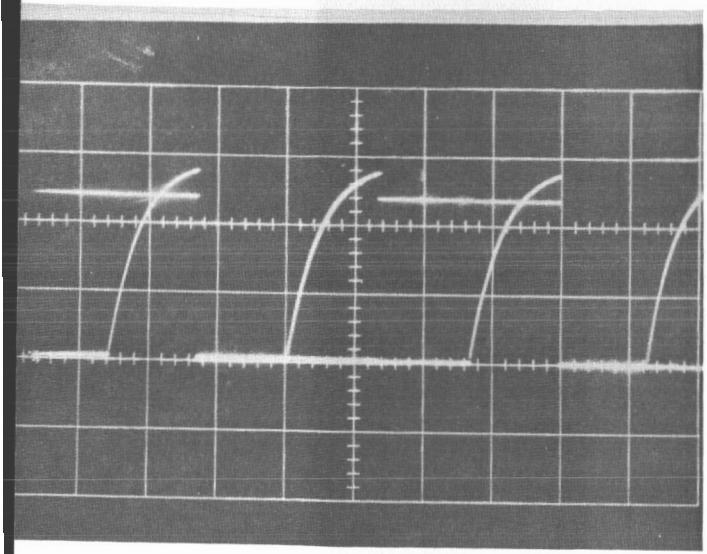
FIGURE	TIME IN TEST	SCOPE SETTING	
		Vert.	Horiz.
10-b	Pre Temp. Soak	2V/cm	0.1 sec/cm
10-c	Initial Post Temp. Soak (After 5 minutes operation)	2V/cm	0.1 sec/cm
10-d	After 4 hours operation	2V/cm	0.1 sec/cm
10-e	After 10 hours operation	2V/cm	0.1 sec/cm
10-f	After 20 hours operation (End of test)	2V/cm	0.1 sec/cm



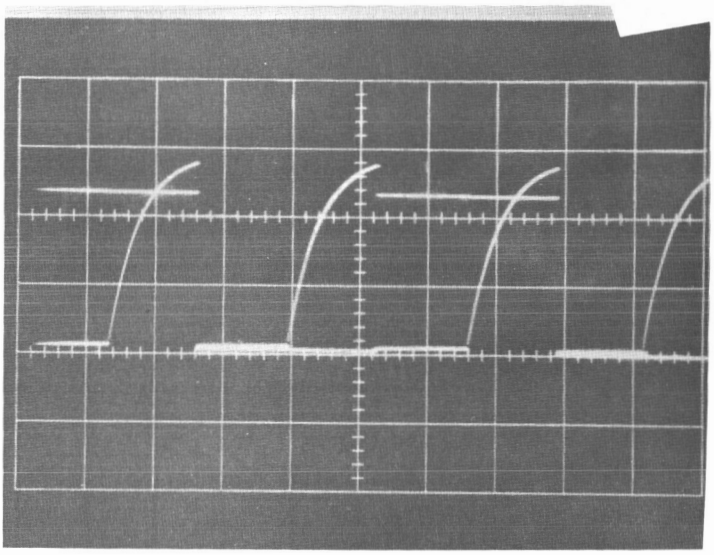
a. TEST SAMPLE BREADBOARD



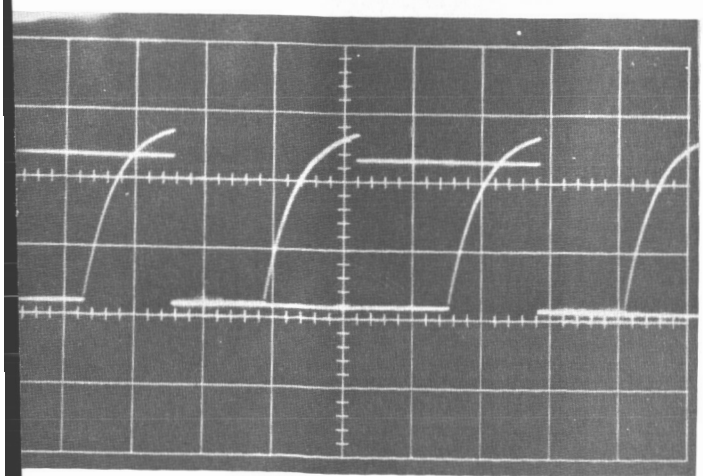
b. PRE TEMP SOAK



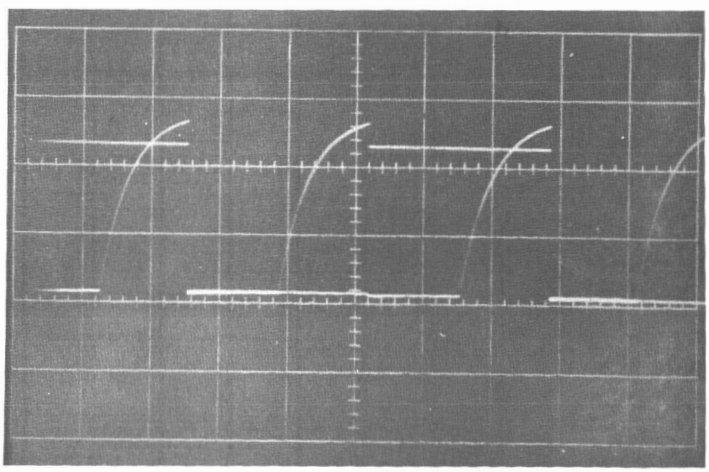
c. INITIAL POST TEMP. SOAK



d. AFTER 4 HOURS OPERATION



e. AFTER 10 HOURS OPERATION



f. AFTER 20 HRS. OPER. END OF TEST

Figure II-10 Test Sample Environmental Tolerance Test Results

There were no detectable changes evidenced in either the pulse shapes, signal levels, or frequency rates of the test circuit waveforms as a result of being subjected to the specified test.

Following satisfactory completion of the breadboard sample, fifty-six printed circuit boards representing "Prime" hardware, were assembled, and 55 of them subsequently operated satisfactorily. The test samples were tested in accordance with the equipment set up of Figure 4-5 of the test plan Revision A, 20 January 1966, Appendix B hereto. The binary counter was excited with a saw tooth signal of 6.4 volts amplitude and a nominal frequency of 8 cps. The output was a square wave with a nominal frequency of 4 cps. and nominal amplitude of 5.5 volts The mean output amplitude of the fifty-five was 5.481 volts (.35% less than the 5.5 volts nominal); the maximum deviation from mean was .181 volts (3.3% of mean); and the spread was .3 volts (5.5% of mean). In the one defective unit, there was no square wave output. This unit was used for destructive bio-assay tests.

B. TASK 2. TEST PROGRAM AND DEMONSTRATION

1) INTEGRATED TEST PLAN

a) Background and History

The test plan for the Test program and demonstration was issued in preliminary form as GE document number 65SD981 on November 5, 1965, and reissued on 20 January 1966 as revision A. The revised test plan, in its entirety, is included in this report as Appendix B.

The preliminary issue of the test plan was found generally acceptable to NASA/LRC with the exception of the bio-assay procedures. Since preparation of the test plan, NASA/LRC had received draft copies of Appendices B and C to the NASA "Sterilization Handbook" on air and fall-out sampling of clean rooms. These documents were reviewed by G.E. at LRC because they were not yet available to Industry. G.E. altered the test plan in accordance with the requested changes and issued it as revision A which was approved by NASA/LRC.

Two further alterations of the program were made: the ethylene oxide treatment in the Analog main chamber was deleted, and the bio-assay procedure was modified. Deletion of the ETO treatment from the planned operation provides a better simulation of the full scale operation as envisioned. The full scale main chamber volume is so large that the use of the ETO gas mix in this chamber is subject to considerable question for economical operation. The change in the bio-assay procedure was made to increase the sensitivity and accuracy of the sterility control specimen assays by using 5 ml aliquots of the sampling diluent instead of plating out the entire 50 ml suspension. By counting ten plates instead of only one large plate, it was possible to obtain a more accurate estimate of the population. The details of these changes are described in Appendix C hereto.

b) Plan Summary

The test plan describes a program to provide the initial demonstration of the technical feasibility of a facility which permits the decontamination and sterilization of spacecraft with capability for subsequent checkout, adjustment, repair, and encapsulation in a biological barrier under sterile conditions. This is demonstrated using a reduced scale analogue of the Assembly/Sterilizer Facility. The test program is composed of three major types of tests:

- Sterilization Verification
- Manipulation Tests
- Feasibility Demonstration

A tabulation of the times involved in performing tasks under the various conditions is shown as a series of ratios in Table D-II of Appendix D. A brief comparison of these ratios leads to the following preliminary conclusions concerning the human factors portion of the test program:

- A wide variety of common assembly tasks can be accomplished in a system with the configuration of the analog. It takes over twice as long, on the average, to perform work in such a system wearing gloves as it does working with bare hands on an open bench.

- Working in a glove box offers few problems that are not present when wearing gloves on an open bench. The major problem is a reduction of visibility when performing some tasks. If the operator is able to manipulate his work so that he can see what he is doing, it takes about the same amount of time to perform equivalent tasks, wearing gloves, whether in or out of the glove box.

- The time to perform a task in the analog is consistently longer than unencumbered on an open bench.

- The percentage increase in the time required to perform a task is, roughly, an increasing function of the complexity of the task.

Similarly, four brief conclusions regarding tool selection and use are included below. The conclusions were based on general observations and tasks which are included with the Human Factor portion of the test program.

- The configuration of common assembly tools is such that no major redesign of shapes is required for their use by a gloved operator.

- Most tools are made of materials that are significantly degraded by exposure to one or more of the three possible treatments; sterilization by steam autoclaving, dry heat sterilization, or Ethylene Oxide/Freon decontamination.

- Several manufacturers offer lines of tools that are, generally, compatible with the sterilization treatments.

- Gripping tools are necessary to compensate for the loss of dexterity caused by wearing gloves. Satisfactory gripping tools are available in standard designs; however, their materials may not be acceptable.

A complete, detailed analysis of the tests which were conducted, the results of the tests, and the conclusions derived therefrom, appear herein in Appendix D.

These tests may be summarized as follows:

The sterilization verification tests assure that the A/S Analog chambers are achieving the required decontamination and sterilization using the prescribed treatments. These tests consist of three complete cycles of operation of the A/S Analog subjecting a total of 150 specially prepared specimens to the treatments of ETO/Freon decontamination, dry heat sterilization. The specimens were stainless steel strips with known, high resident populations of viable micro-organisms.

The manipulation tests consisted of a limited human factor test to determine the limitations imposed on a worker performing assigned tasks in the A/S Analog, and problems resulting from this work environment. In addition, tools suitable for sterile assembly and sterilization facility procedures were investigated.

The feasibility demonstration consisted of the performance of five cycles of A/S Analog operation including decontamination, sterilization and sterile checkout, repair, assembly, packaging and recycle repair of a special component simulating typical spacecraft hardware. A total of 50 of these components were available for this program. However, 5 were shipped to NASA Langley for independent testing. In addition, 172 biologically seeded stainless steel strips were processed during these cycles to broaden the biological base of the demonstration.

2) MANIPULATION TESTS

The Manipulation tests were primarily a human factors study to investigate the limitations imposed on an operator by the gloves, the pressure environment, and his hand and arm movements within the A/S Analog. A second objective of the tests was to make a preliminary investigation into tools suitable for sterile assembly procedures.

In order to carry out the Manipulation Tests efficiently, the tests were split into three phases. The purpose of the phases was to allow a wider range of work tasks to be studied than could be accomplished by working only on the test sample and to permit a majority of the test tasks to be performed outside the A/S Analog. Testing outside the Analog was necessary because time available for Manipulation Testing within the Analog was limited by other tests being run as part of the A/S Test Program. To facilitate testing outside of the Analog, the General Electric Company constructed an Assembly/Sterilizer Analog panel simulator (mock-up) using equivalent gloves and the same geometry as the Analog front panel. The mock-up is illustrated in Figure II-11. The Analog is illustrated in Figures II-12 through II-15.

Phase 1 consisted of assembly of typical flight hardware under unconstrained open-bench conditions (Condition A); while wearing gloves at an open bench (Condition B); and while working through a simulated glove box panel front (Condition C). Phase 2 was conducted under the same conditions but using the test sample as a test piece. Phase 3 involved assembly of both flight hardware and test samples within the Assembly/Sterilizer Analog (Condition D).

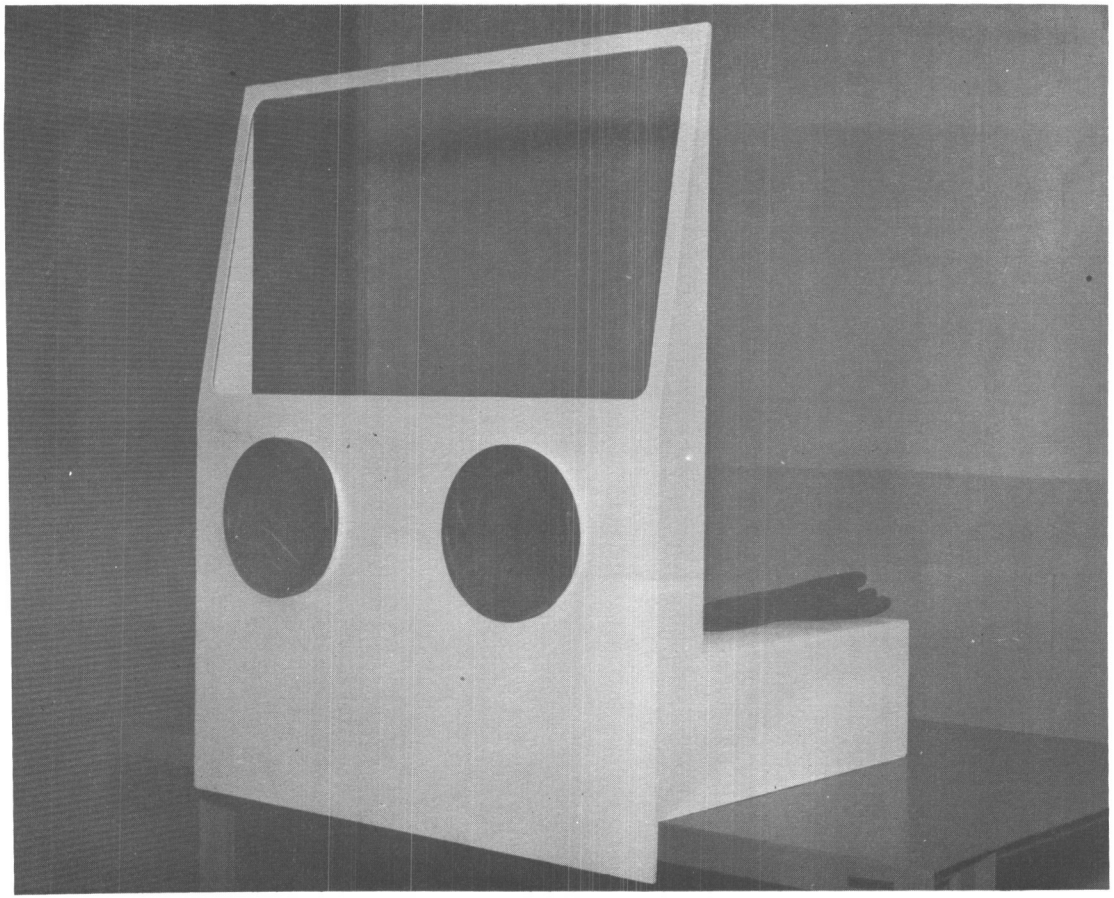


Figure II-11 Assembly/Sterilizer Analog Simulator "Mock-Up"

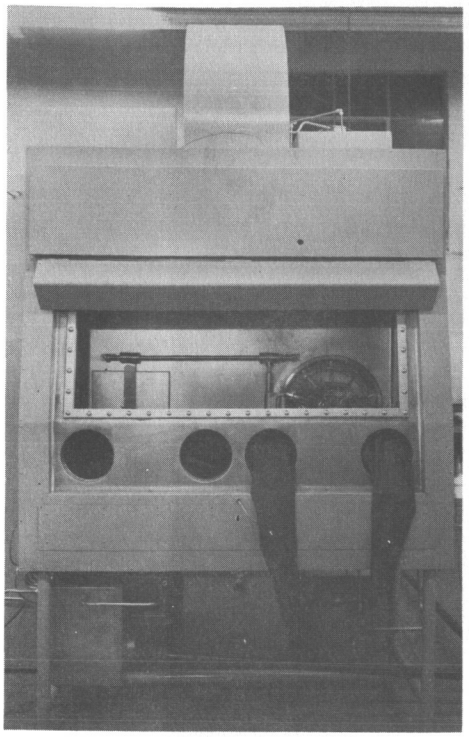


FIGURE II-12
FRONT VIEW

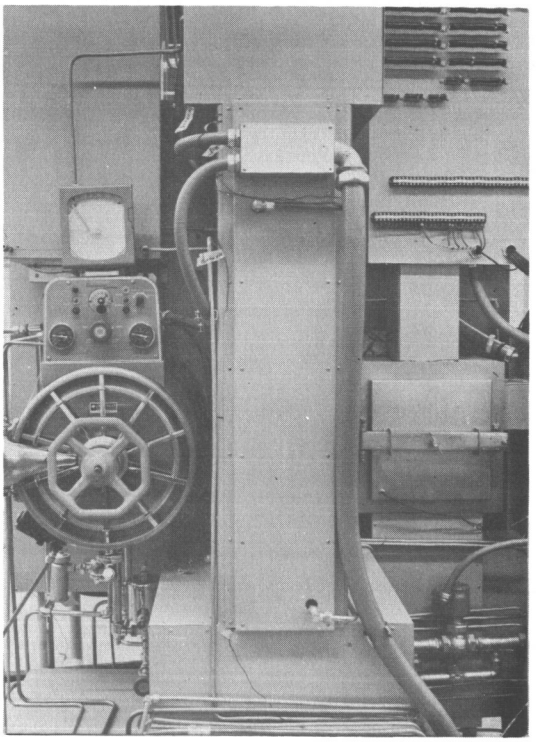


FIGURE II-13
REAR VIEW

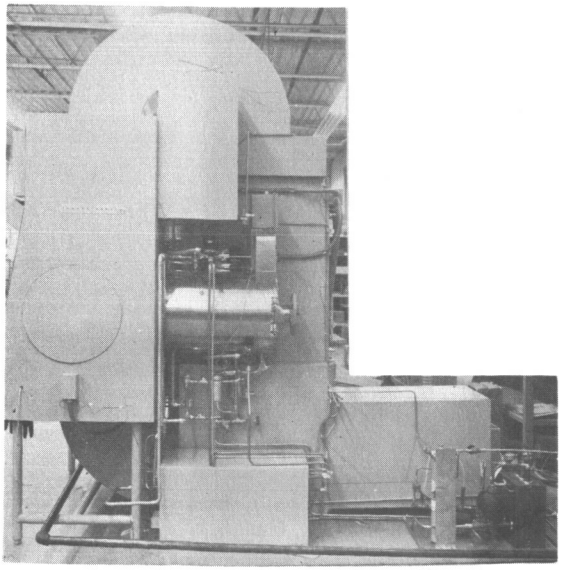


FIGURE II-14
END VIEW

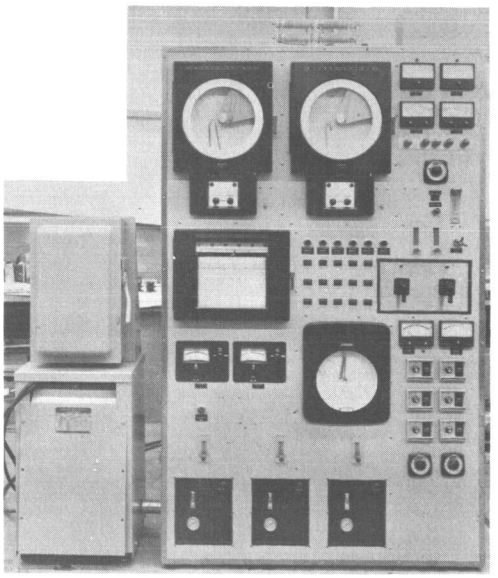


FIGURE II-15
CONSOLE

3) STERILITY VERIFICATION TESTS

Sterility verification testing was included in the test plan to verify that the Assembly/Sterilizer Analog could satisfactorily perform prescribed decontamination and sterilization treatments. The test consisted of three performances of a sterilization verification cycle. Stainless steel strips, measuring 1" x 2" x 0.06", and incubated with populations of known viable organisms, were subjected to ETO/FREON 12 decontamination, dry heat sterilization, wet heat sterilization, and various combinations of all three. The test results provided a base line condition for assessment of the collected data from the subsequent feasibility demonstration out tests.

After each sterilization or decontamination step, sample sterility control specimens were placed in sterile media, and incubated according to the biological procedure outlined in Appendix B. Following incubation, counts were made, the results of which appear in section B of Task 3 herein.

A step by step breakdown of the verification cycle and the revised material flow chart appear in Appendix C.

Figure II-16 is a time vs. temperature chart of the sterility verification cycle.

4) DEMONSTRATION TESTS

The objective of the demonstration tests was to provide laboratory verification of the feasibility of the Assembly/Sterilizer concept for a system used to support an interplanteary spacecraft sterilization program. The tests consisted of performing typical sterilization, assembly, checkout, and packaging tasks on test samples. A prime requirement was to perform only those operations in the Assembly/Sterilizer which would be representative of those performed in a full scale facility.

Five demonstration cycles were performed, two of which were designed to demonstrate the feasibility of substituting repair parts on a previously sterilized assembly. The remaining three cycles were considered "normal" in that no malfunction of processed equipment was assumed. The number of test samples used for each cycle varied between eight and fourteen, and permitted the destruction bio-assay of eight test samples after each cycle. The temperatures of all the test samples were monitored during testing.

The test samples were subjected to performance tests before processing in the demonstration cycles. These tests were repeated prior to sterilization, after sterilization, and at the end of a twenty hour life test. No significant changes in pulse shapes, signal levels, or frequency rates of the test samples resulted from ETO decontamination, dry heat sterilization, or the life test. This was the expected result based on the experience from the qualification testing previously performed on the test sample breadboard.

A summary of the distinguishing features of the five demonstration cycles is included in Table II-II.

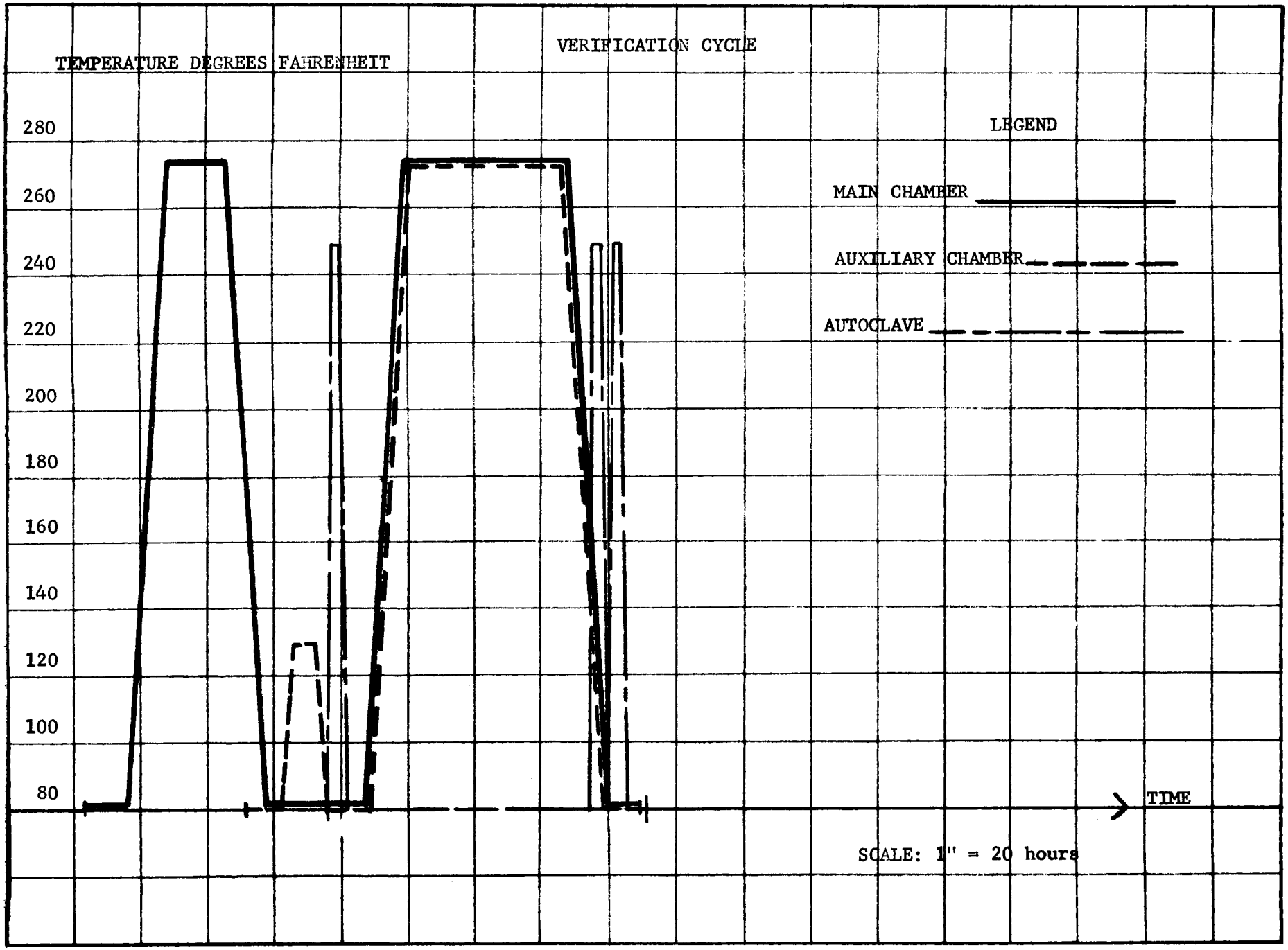


Figure II-16 Time vs. Temperature Chart

TABLE II-II - SUMMARY OF FEASIBILITY DEMONSTRATION TEST CYCLES

CYCLE NUMBER	DEMONSTRATION CYCLE	TOTAL CYCLE DURATION (HR.)	QUANTITY OF TEST SAMPLES	QUANTITY OF STERILITY CONTROL SPECIMENS	DECONTAMINATION TREATMENT			STERILIZATION TREATMENT			COMMENTS
					MEDIUM	TEMPERATURE °C/°F	DURATION (HR.)	TYPE OF HEAT	TEMPERATURE °C/°F	DURATION (HR.)	
1	"Normal" cycle	89.75	9	27	ETO *Dry Heat	54/130 135/275	2 5	Dry	135/275	24	Use autoclavefor bioassay material sterilization (wet load) ETO decontamination in auxiliary chamber and dry heat sterilization in main chamber.
2	Repair Cycle	90.75	8	16	ETO *DRY Heat	54/130 135/275	2 5	Dry Dry	135/275 135/275	24 24	Use autoclave for bioassay material sterilization (wet load). ETO decontamination in auxiliary chamber, dry heat sterilization of 4 test samples in main chamber, and 4 test samples in dry-heat pass-through. Printed circuit boards introduced through pass-through are used on bases sterilized in main chamber and vice versa.
3	"Normal" cycle	91.25	14	42	ETO *Dry Heat	54/130 135/275	2 5	Dry	135/275	24	This cycle differs from cycle No. 1 in that 5 of the test samples are placed in sterile containers after the 20 hour life test.
4	Re-cycle Repair	92.0	5 5	20 5	ETO *Dry Heat	54/130 135/375	2 5	Dry Wet	135/275 121/250	24 ½	Sterilize main chamber before introducing test samples. Pass 5 samples through dry heat ; and 5 through autoclave, surface sterilizing containers. Printed circuit boards introduced through pass-through are used on bases introduced through autoclave and vice versa.
5.	"Normal" Cycle	89.75	9	27	ETO *Dry Heat	54/130 135/275	2 5	Dry	135/275	24	This cycle is an exact duplicate of Cycle No. 1

* DRY HEAT Decontamination of main chamber.

In the normal cycle (Cycles 1, 3 and 5) the primary objective was to serve as a "control" so that significant changes in the operation of the equipment could be detected.

In the repair cycle (#2), it was assumed that all of the test samples in the main chamber had an indicated malfunction in the post sterilization checkout. A simulated repair was effected by introducing an equal quantity of test samples through the auxiliary chamber. The electronics from the replacement units were substituted for the electronics on the failed units and vice versa. The purpose of this test was to demonstrate that repairs can be made to a sterile unit without violating sterile conditions or re-sterilizing the failed unit.

The re-cycle repair cycle (#4) was similar to the repair cycle except that it involved the introduction of a sterile test sample, in a container, into the sterile main chamber thus simulating repair of an on-pad failure of a sterile spacecraft. Because of the small physical size of the test sample and its container with the consequent thermal lag, the autoclave was used to pass the sterile test samples into the main chamber. This permitted rapid surface sterilization of the test sample container and greatly reduced the probability of killing any viable organisms which might be on the test sample itself. This concept was verified by the simulatneous processing of S/C specimens in the same manner. After the recycled test samples were introduced to the main chamber, a repair was simulated as in Cycle No. 2 The results obtained by performing bio-assays on the sterility control specimens were satisfactory. A complete, detailed analysis of the test results appears herein in Task 3.

C. TASK 3. BIO-ASSAY

The objectives of the bio-assay portion of the Assembly/Sterilizer program were met satisfactorily. The data showed that ethylene oxide decontamination reduced the microbial populations by at least three and as much as eight decades, sterilization was effected by the dry heat and steam cycles uniformly throughout the chambers, and there was no incidence of recontamination of either the assembled Test Samples or the Sterility Control Specimens during any of the cycles.

1) BIO-ASSAY PLANNING

a) Experimental Rationale

The bio-assay test plan was devised to monitor the microbiological functions of the Assembly/Sterilizer analog, its efficacy in decontaminating, sterilizing, and maintaining sterility of spacetype hardware through assembly and testing. The specific objectives of the bio-assay tasks performed were to demonstrate the effectiveness of the ethylene oxide decontamination cycle in reducing microbial populations, to confirm temperature measurements indicating lethality of dry heat sterilizing cycles, and to detect microbial contamination if introduced to the test samples or sterility control specimens subsequent to sterilization.

Because it is anticipated that major systems of spacecraft processed according to the Assembly/Sterilizer concept would be assembled in bio-clean facilities prior to decontamination and sterilization in the partially assembled state, the test samples were put through a similar process. Structural parts were assembled under normal manufacturing conditions and decontaminated by heating. Electronic piece parts were cleaned and bagged prior to assembly on a clean bench, and all parts were bagged for storage until they were used in the analog. This entire process was monitored according to procedures employed throughout NASA* to enable comparison of the results obtained with those of other investigators.

Because the magnitude of the population anticipated on the assemblies and piece parts was small, it was decided to supplement the resident populations with large numbers of dry-heat resistant bacterial spores.

The relatively small numbers of samples would have limited the obtainable data; therefore, additional spore preparations were employed in large numbers, enabling more complete distribution throughout the analog and increasing the confidence in the data obtained. In addition to temperature, the resistance of spores is a function of the atmospheric composition, particularly oxygen and water vapor concentrations. In a system as geometrically complex as the analog, it was necessary to demonstrate that the local environments within the chambers were sufficiently uniform to achieve sterilization reliably. This is effectively and conveniently demonstrated with microbiological controls.

*Paragraph C, "Method of Sampling Fallout Contamination Onto Surfaces" - Interim Requirements for Bio-Clean Facilities, Appendix B, Hq, National Aeronautics and Space Administration.

b) Procedure Selection

(1) Test Sample Bio-assay

(a) Bio-assay of Fabrication Facilities

Standard NASA* procedures were chosen for bio-assy of the clean bench and the clean room housing the analog. Reynier samples and fallout strips were employed as described in Appendix B.

(b) Bio-assy of Test Sample Hardware

Piece parts and sub-assemblies were bio-assayed for surface microbial population only consistent with the intent of the program to demonstrate asceptic assembly and testing. The assays were performed in accordance with standard NASA* procedures as described in Appendix B.

(2) Preparations for Sterility Verification and Demonstration Cycles

(a) Preparation of Sterility Control Specimens

Spores of Bacillus subtilis var. niger were chosen for evaluation of the ethylene oxide decontamination and dry heat cycles because they exhibit relatively high resistance to these agents, produce colonies of characteristic morphology, and have been employed widely as sterilization indicators. Spores of Bacillus stearothermophilus were chosen for application in steam sterilization as they are standards in the industry and possess exceptionally high moist heat resistance. Both organisms were employed on stainless steel strips in populations of approximately 10^6, 10^8, and 10^9 per strip as described in Appendix B.

(b) Inoculation of the Test Samples

Test samples were inoculated with 10^8 spores of B. subtilis var. niger to supplement the resident microbial population.

(3) Sterilization Verification Cycles

The principal purpose of the Sterilization Verification Cycles was to demonstrate the readiness of the various chambers of the analog to perform the decontamination and sterilization tasks before proceeding with the demonstration cycles. A second objective was to check out the bio-assay procedures that would be employed in the demonstration cycles.

The normal cycle of ethylene oxide decontamination, sterilization, manipulation and assay was employed. In evaluating decontamination, samples were removed in sterile containers and bio-assayed by normal viable counting procedures to determine the reduction in population attributable

* Paragraph C, "Method of Sampling Fallout Contamination Onto Surfaces" - Interim Requirements for Bio-Clean Facilities, Appendix B, Hq, National Aeronautics and Space Administration.

to the ethylene oxide environment. The specimens to be subjected to dry heat were sealed in jars during the decontamination cycle to protect them from the ethylene oxide environment. Sufficient specimens were included that five from each group of sealed specimens were removed for assay before the heat cycles to verify the viable population, demonstrating the integrity of the seal. The remainder were deployed throughout the chambers for dry heat sterilization, and finally assayed for sterility in aerobic broth medium. The details of the cycles can be found in Appendices B and C.

(4) Demonstration Cycles

The principal purpose of the demonstration cycles was to demonstrate the capability of the various chambers of the analog to perform the decontamination and sterilization. These were, to decontaminate and sterilize electronic piece parts and structural sub assemblies, to demonstrate the effectiveness of each cycle, and to show they could be built, tested, repaired and maintained aseptically for prolonged periods of time. This was accomplished through the use of a multitude of control specimens as noted in the test plans in Appendices B and C.

Control specimens consisting of previously inoculated stainless steel strips were utilized to demonstrate the effect of each cycle with all bio-assaying accomplished in the analog to eliminate the possibility of contamination due to handling.

As an additional control, the electronic piece parts and portions of materials of the structural sub assemblies were individually seeded to exhibit growth. This demonstrated that the pieces in themselves were neither bacteriostatic nor bacteriocidal by nature of their chemical composition.

c) Evaluation of Results

Performance of the Sterilization Verification Cycles and the Demonstration Cycles without a single instance of failure in sterilization or detected breach of sterility is essential to the acceptance of the Assembly/Sterilizer as a valid concept. The degree of assurance required by the Planetary Quarantine requirements for interplanetary exploration that sterility is not violated cannot practically be developed solely by experimentation because of the number of experiments required. Such assurance will have to be gained analytically. Consequently, results indicating no violation of sterility cannot of themselves confirm the feasibility of the concept, but a single violation of sterility is an unequivocal indicator of a flaw in the system.

Caution must be exercised in applying the effectiveness of ethylene oxide decontamination experienced in the analog system to other ethylene oxide systems. The conditions attained in the decontamination mode of operation were not optimal for ethylene oxide decontamination as unexplained variations in ethylene oxide, freon and air concentration occurred throughout the program.

The microbial population associated with the clean room in which the Assembly/Sterilizer is located was examined in an attempt to establish any specific trends for an analysis. The fallout samples present data which is so widely spread that no pattern is evident. Larger sample quantities at more frequent intervals would have to be taken to develop a pattern of accumulation and die off.

The airborne population density average was $.21/ft^3$ with an average upstream population of $.18/ft^3$ and downstream population of $.24/ft^3$.

The laminar flow clean bench exhibited an airborne population of 1.3×10^{-2} counts/ft^3 and a fallout population ranging from $0/ft^2$ to $720/ft^2$ over a two week period. With an average of:

75	Aerobic non heat shocked	/ FT^2 /	2 Weeks
30	Aerobic heat shocked	/ " /	" "
0	Anaerobic heat shocked	/ " /	" "
0	Anearobic non heat shocked	/ " /	" "

The microbial population on the test samples was found to be very low as anticipated. The average count for the components and piece parts of an assembly was 10.2 - aerobic nonsporeformers; 11.0 - aerobic sporeformers; 4 - anaerobic nonsporeformers and 0 - anaerobic sporeformers.

2) BIO-ASSAY PERFORMANCE AND RESULTS

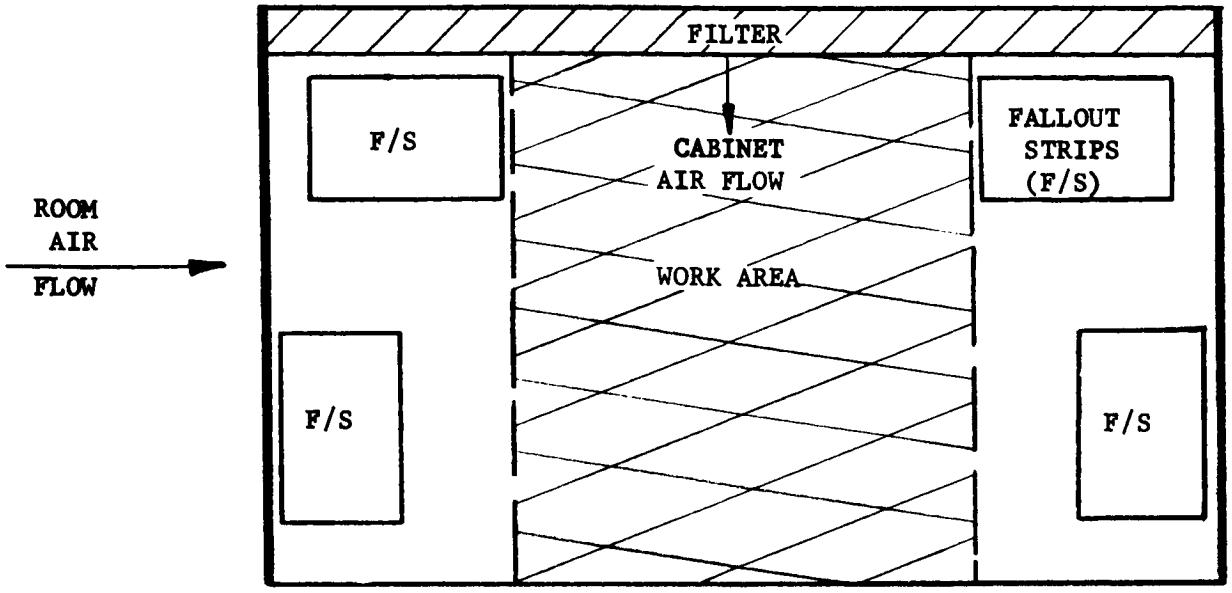
a) Test Sample Bio-assay

(1) Air Sample Bio-assay of the test sample fabrication facility environment in and about the class 100 laminar flow bench.

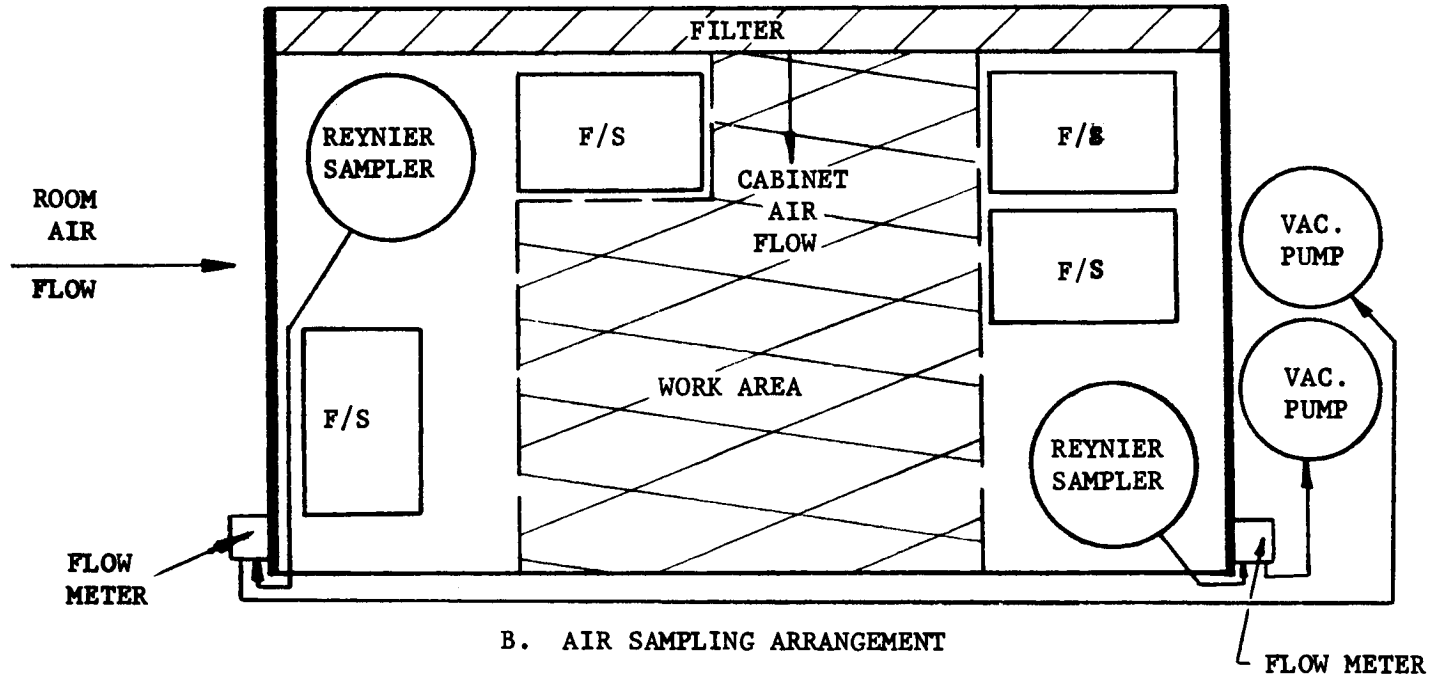
Thirty-six hours of air sampling were performed: 4 hours of room environment sampling and 32 hours of clean bench sampling. The petri dishes from the Reynier samplers were prepared and incubated in accordance with the test plan of Appendix B. A total of 25 colonies resulted from the incubation of the bench air samples. Since the rate of sampling is one cfm, this gives an average colony population in the bench air of $25/(32 \times 60) = 1.3 \times 10^{-2}$ colonies per cubic feet. The data from which this average was derived are presented in Table II-III.

Twenty-four fallout strips were also placed in the laminar flow bench, and removed for bio-assay at periodic intervals (4, 6, 12, & 17 days). The bio-assay of these strips gave computed average fallout accumulations of one hundred colonies per square foot for aerobic, non-heat shocked, sixty colonies per square foot for aerobic heat shocked, and zero colonies per square foot for anaerobic heat shocked and non-heat shocked. These averages are derived by weighting the data of table II-IV by the number of strips for each sample. It should be noted that the data in the tables represents a total of only seven colonies which resulted from the assay of the twenty-four strips. This very low count is due to the fact that the environment sampled is a bio-clean bench in a quasi-clean room. The resultant count is too low to try to draw statistically significant conclusions from the data.

Figure II-17 shows the placement of the Reynier samplers and fallout strip trays on the bench during normal working conditions and during air sampling.



A. NORMAL WORKING CONDITIONS



B. AIR SAMPLING ARRANGEMENT

FIGURE II - 17

PLACEMENT OF FALLOUT SAMPLES AND

REYNIER SAMPLERS ON CLEAN BENCH

TABLE II-III

BIO-ASSAY RESULTS OF AIR SAMPLES FROM CLEAN BENCH (Note 3)

SAMPLE #	POSITION	DATE	TIME	Colonies/ft^3 - 5 minutes segments (Note 4)											
				1	2	3	4	5	6	7	8	9	10	11	12
1(Note 1)	Clean room upstream	1/21	PM	0.6	0.2	0.2	0.6	0.2	0	0	0	0	0.4	0.2	0
2 "	Clean room downstream	1/21	PM	3.8	0.6	0.4	0	0.4	0.6	0.4	0.2	0.2	0.4	0	0.4
3 "	Clean room upstream	1/24	AM	0.6	0.2	0.2	0.2	0.2	0.6	0	0	0.2	0	0	0.2
4 "	Clean room downstream	1/24	AM	0.6	0.2	0.2	0	0	0	0.2	0.2	0.2	0	0	0
5(Note 2)	F	1/25	AM	0	0	0	0	0	0	0	0	0	0	0	0
6	0	1/25	AM	0	0	0	0	0	0	0	0	0	0	0	0
7	F	1/26	AM	0	0.4	0	0	0	0	0	0	0	0	0	0
8	0	1/26	AM	0	0	0	0	0	0	0	0.2	0	0	0	0
9	F	1/26	PM	0	0	0	0	0	0	0	0	0	0	0	0
10	0	1/26	PM	0	0	0	0	0	0	0	0	0.2	0	0	0
11	F	1/27	AM	0	0.2	0	0	0	0	0	0	0.4	0	0.4	0
12	0	1/27	AM	0	0	0	0	0	0	0	0	0	0	0	0
13	F	1/27	PM	0	0	0	0	0	0	0	0	0	0	0	0
14	0	1/27	PM	0	0	0	0	0	0	0	0	0.2	0	0	0
15	F	1/28	AM	0	0	0	0	0	0	0	0	0	0	0	0
16	0	1/28	AM	0	0	0	0	0.2	0	0	0	0	0	0	0
17	F	1/31	AM	0.2	0	0	0	0	0.2	0	0	0.2	0	0	0
18	0	1/31	AM	0.2	0	0	0	0	0	0	0	0	0	0	0
19	F	2/2	PM	0	0	0	0	0.2	0	0	0	0	0	0	0
20	0	2/2	PM	0	0	0	0	0	0	0	0	0	0	0	0

TABLE II-III (Continued)

SAMPLE #	POSITION	DATE	TIME	Colonies/ft^3 - 5 minute segments											
				1	2	3	4	5	6	7	8	9	10	11	12
21	F	2/3	AM	0	0	0	0	0	0	0	0	0	0	0	0
22	0	2/3	AM	0	0	0	0	0	0	0	0	0	0	0	0
23	F	2/3	PM	0	0	0	0	0	0	0	0	0	0	0	0
24	0	2/3	PM	0.2	0	0	0	0	0	0	0	0	0	0	0
25	F	2/4	AM	0	0	0	0	0	0	0	0	0	0	0	0
26	0	2/4	AM	0	0	0	0	0	0	0	0	0	0	0	0
27	F	2/4	PM	0	0	0	0	0	0	0	0	0	0	0	0
28	0	2/4	PM	0	0	0	0	0	0	0	0	0	0	0	0
29	F	2/7	AM	0	0	0	0	0	0	0	0	0	0	0.2	0.2
30	0	2/7	AM	0	0	0	0	0	0	0	0	0	0	0	0
31	F	2/7	PM	0	0	0	0	0	0	0	0	0	0	0	0.2
32	0	2/7	PM	0	0	0	0	0.2	0	0	0	0	0.2	0	0
33	F	2/8	AM	0	0	0	0	0	0	0	0	0	0	0	0
34	0	2/8	AM	0	0	0	0	0	0	0	0	0	0	0	0
35	F	2/8	PM	0	0.2	0	0	0	0.2	0	0	0	0	0	0
36	0	2/8	PM	0	0	0	0.2	0	0	0	0	0	0	0	0

NOTES:

(1) These results represent initial air sampling taken in the room (a quasi - laminar flow room) upstream and downstream of the bench to determine background contamination in the room for comparison with the clean bench environment. The remaining results are from the clean bench environment.

(2) Sampler positions on the clean bench are indicated by: F - next to fiter wall in rear corner
0 - next to bench opening diagonally opposite F

TABLE II-III (Continued)

NOTES (Continued)

(3) All samples were collected by solid impingement onto trypticase soy agar in Reynier samplers.

(4) Sample computation for sample #1, time segment 1:

Number of colonies = 3
Duration of segment = 5 minutes
Air sampling Rate = 1 cfm

$$\text{Colonies/ft}^3 = \frac{\text{number of colonies}}{\text{Duration of segment x air sampling rate}}$$

$$= \frac{3 \text{ colonies}}{5 \text{ min} \times 1 \text{ cfm}} = .6 \text{ colonies/ft}^3$$

TABLE II-IV

BIO-ASSAY RESULTS OF FALLOUT SAMPLES ON CLEAN BENCH

Date (Note 1)	COLONIES PER SQUARE FOOT (Notes 4,5,&6) AEROBIC		ANAEROBIC	
	Non-heat Shocked	Heat-Shocked	Non-Heat Shocked	Heat-Shocked
1/25/66 (Note 2)	240	120	0	0
1/27/66 (Note 3)	0	---	0	---
2/3/66 (Note 3)	0	---	0	---
2/7/66	60	0	0	0

NOTES:

(1) Strips set out on 1/21/66

(2) Average of colonies from 6 fallout strips - rinse method

(3) Average of colonies from 3 fallout strips per incubation condition - agar immersion method

(4) All controls negative

(5) Sample computation for 1/25/66 aerobic non-heat shocked

Strip size - 1" x 2" = 2 $in.^2$

Number of strips = 6

Total strip area = 6 x 2 $in.^2$ = 1/12 $ft.^2$

Aliquot fraction = 1/5

Number of colonies = 4

$$\text{Colonies/ft.}^2 = \frac{\text{Number of colonies}}{\text{Aliquot fraction x Total strip area}} = \frac{4}{(1/5)\times(1/12)} =$$

240 colonies/$ft.^2$

(6) Sample computation for aerobic non-heat shocked average colonies per square foot:

$$\text{Colonies/ft.}^2 = \frac{\sum(\text{colonies/ft.}^2 \text{ for each sample}) \times (\text{numbers of strips})}{\text{Total number of strips}}$$

$$= \frac{240 \times 6 + 0 \times 3 + 0 \times 3 + 60 \times 6}{18} = \frac{1800}{18} = 100 \text{ colonies/ft.}^2$$

(2) Bio-Assay of Test Sample Hardware

Bio-assay of the test sample consisted of assaying electronic piece parts, wired and unwired printed circuit boards, and base assemblies in accordance with the test plan (except as noted below). Ninety-five piece parts, representing sets of parts for five test samples were assayed. There were a total of 18 colonies observed giving an overall average of 0.19 colonies per part. The data from this assay are presented in Table II-V.

TABLE II-V

BIO-ASSAY RESULTS ON ELECTRONIC PIECE PARTS

Part Type	Sample No.	Parts/ Sample	Colonies/Sample (Note 1)* Non-Heat Shocked Aerobic	Non-Heat Shocked Anaerobic (Note 2)	Heat Shocked Aerobic	Heat Shocked Anaerobic (Note 2)
Resistor	1	8	0	0	0	0
	2	8	0	0	0	0
	3	8	0	0	1	0
	4	8	0	0	2	0
	5	8	0	0	0	0
Terminal	1	5	0	0	0	0
	2	5	0	0	0	1
	3	5	0	0	0	0
	4	5	0	0	0	0
	5	5	5	0	2	0
Transistor	1	2	0	0	0	0
	2	2	0	0	0	0
	3	2	1	0	0	0
	4	2	1	0	0	0
	5	2	1	0	0	0
Diode	1	2	0	0	0	0
	2	2	0	0	0	0
	3	2	0	0	0	0
	4	2	0	0	1	0
	5	2	0	0	0	0
Capacitor	1	2	1	1	0	0
	2	2	0	0	0	0
	3	2	0	0	0	0
	4	2	0	0	0	0
	5	2	1	0	0	0
Sterility Control		1	0	0	0	0
(Stainless Steel Strip)		1	0	0	0	0
		1	0	0	0	

*NOTES ON NEXT PAGE

NOTES:

(1) For each part type: Colonies/part (average) = $\frac{\text{Colonies/Sample}}{\text{Parts/Sample}}$

(2) Culture Controls on Anaerobic Incubation
C. sporogenes - all positive
A. faecalis - all negative

During the seventy two hours of incubation for the piece parts, corrosion occurred on some of the part types. It appeared on the zinc plated copper leads of the resistors and on the underside of the transistor bodies. Figures II-18, 19, and 20 show this corrosion. It was verified that this was corrosion, and not microbial growth, by transferring samples of the corrosive products to fresh media and then incubating. The results were negative. It was further confirmed that this growth was not inhibitory to microbial growth by streaking the face of representative plates with A. faecalis and then incubating the plates. The corrosive products were observed to cause no detectable zone of inhibition. The results from this latter test are shown in Figure II-20

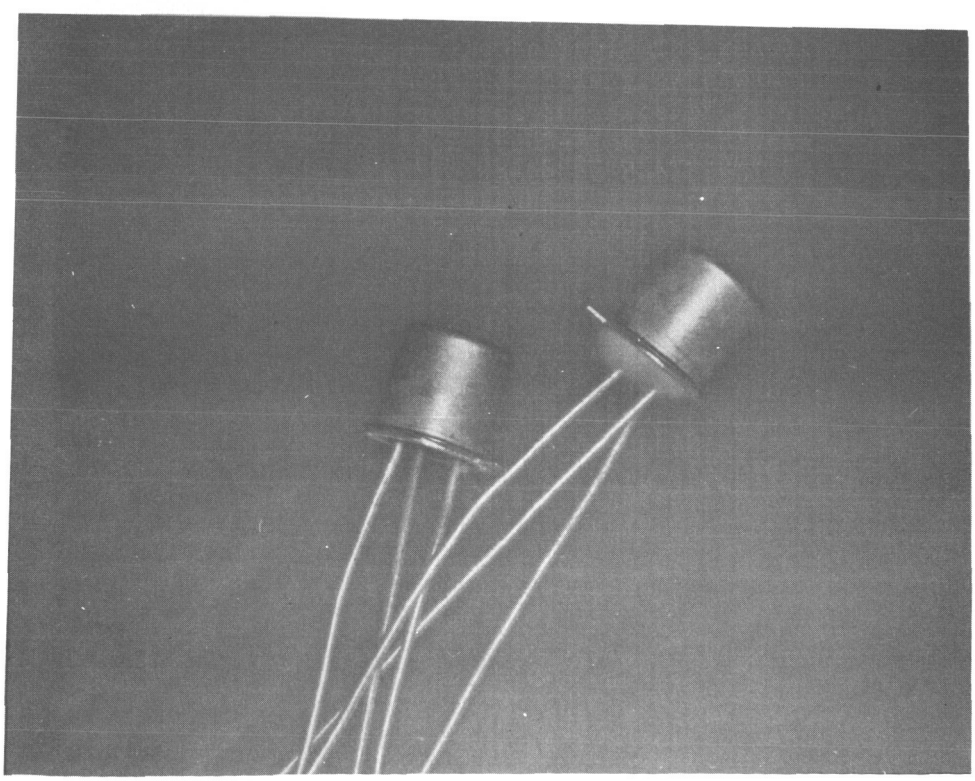


FIGURE II-18

TYPICAL TRANSISTOR ASSAY PLATE

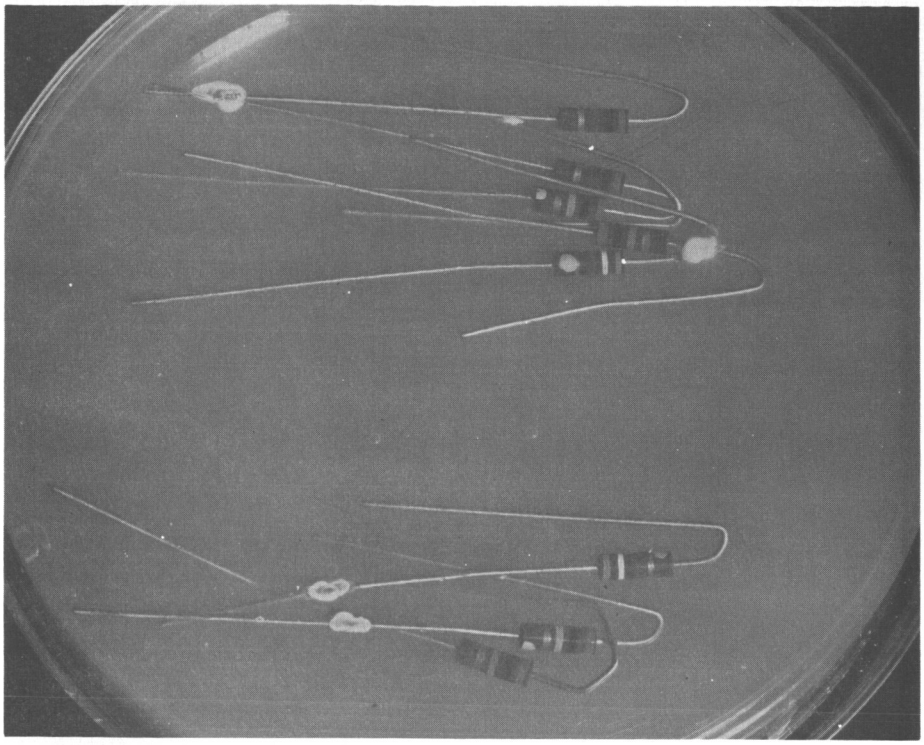


FIGURE II-19
TYPICAL RESISTOR ASSAY PLATE

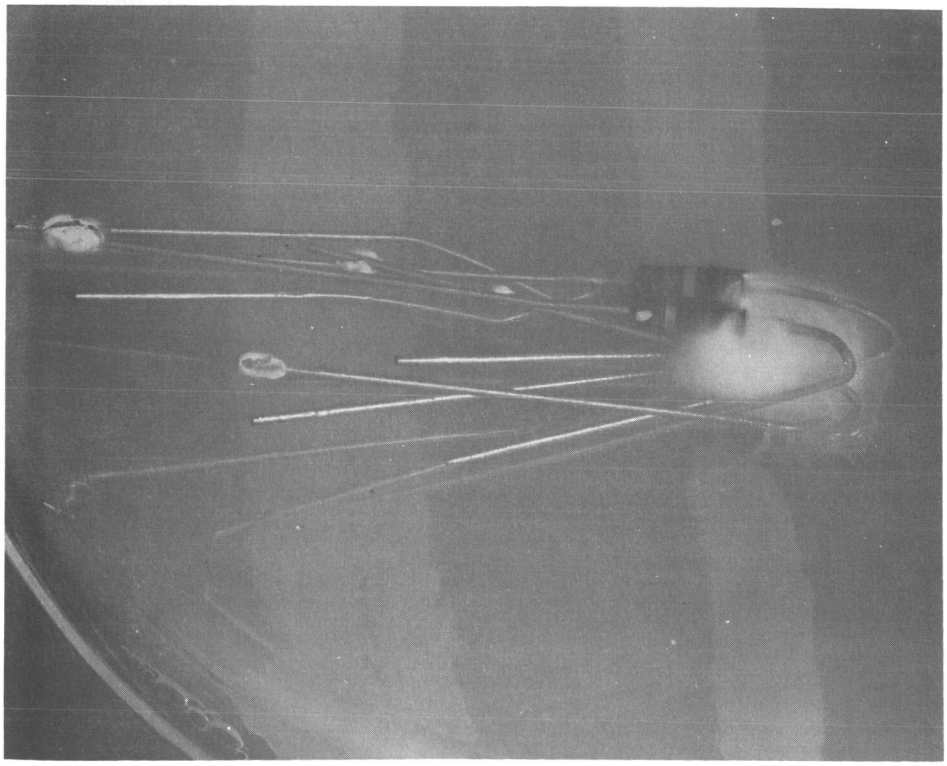


FIGURE II-20
TYPICAL RESISTOR ASSAY PLATE
WITH INHIBITION TEST STREAKS

Five unwired printed circuit boards were assayed. Each board was broken into three pieces so that they could be placed in tubes for washing in sterile 1% peptone water. The data from the assay is presented in Table II-VI. The overall averages of colonies per board are non-heat shocked aerobic 6 per board, and anaerobic 2 per board, and heat shocked aerobic 48.5 per board and anaerobic one per board. (Each board has a free surface area of about 14 square inches).

TABLE II-VI

BIO-ASSAY RESULTS OF CIRCUIT BOARDS (Note 2)

Circuit Board #	Non-Heat Shocked Rinse Aliquot Aerobic	Non-Heat Shocked Rinse Aliquot Anaerobic (Note 1)	Heat Shocked Rinse Aliquot Aerobic	Heat Shocked Rinse Aliquot Anaerobic (Note 1)	Heat Shocked Plated Pieces Aerobic
1	2 1	1 1	1 3 4	0 1	6
2	2 1	0 0	1 3 3	0 0	1
3	0 0	0 0	0 0 0	0 0	7
4	0 0	0 0	1 1 0	0 0	2
5	0 0	0 0	3 17 30	0 0	3
Sterility Control	0 0	0 0	0 0 0	0 0	0

NOTES:

(1) Culture controls on anaerobic incubation
C. sporogenes - all positive
A. faecalis - all negative

(2) Each of the numbers for aliquots represents a 1/10 aliquot. Thus a sample calculation for the average colonies per board for Aerobic heat shocked is:
Number of boards - 5
Total count from rinse aliquots - 67
Aliquot fraction - 3/10
Total count from plated parts - 19

$$\text{Colonies/board} = \frac{\text{Total count from aliquots}}{\text{Number of boards} \times \text{Aliquot fraction}} + \frac{\text{Total count from plated parts}}{\text{Number of boards}}$$

$$= \frac{67}{5 \times 3/10} + \frac{19}{5} = 48.5 \text{ colonies/board}$$

Five wired test sample printed circuit boards (SN 8, 19, 38, 51 and 56) were bio-assayed to define the pre-test condition for the test cycles of Task 2. With the exception of SN 56, the total reduced count was 11 aerobic non-heat-shocked organisms on four samples with a total surface area of about 80 square inches. This gives an average surface count of 20 organisms per square foot. Culturing of the aliquots from SN 56 resulted in populations too numerous to count* The data from the assay are contained in Table II-VII.

TABLE II-VII ASSEMBLED PRINTED CIRCUIT BOARD

MICROBIOLOGICAL ASSAYS
(Notes 1 & 2)

Sample Serial No.	Non-Heat Shocked Aerobic	Non-Heat Shocked Anerobic	Heat Shocked Aerobic	Heat Shocked Anaerobic	Plated Parts Aerobic Non-Heat Shocked
8	1 (5)	0	0	0	0
19	1 (5)	0	0	0	0
38	0	0	0	0	0
51	0	0	0	0	1
56	TNTC	0	TNTC	0	TNTC
CONTROL	0	0	0	0	0

NOTES: 1. Control Samples on Anaerobic incubation

C sporogenes	Pos.
A faecalis	Neg.

2. The numbers 1 (5) represent a count of one in a 20% aliquot giving a reduced count of 5.

* Analysis of the growth from the assay of sample SN 56 has shown it to be morphologically identical with B. subtillis var. niger which, during the period of these assays, was being used in massive quantities in the biological laboratory. Thus, this growth is attributed to laboratory contamination of the specimen.

Five test sample base assemblies were bio-assayed. To handle, cut, and pull apart the assemblies, sterile pliers, forceps, and shears were used. Component #SN-45 was cut randomly into pieces small enough to fit into peptone water bottles, and attempts were made to flatten out the carious pieces so as to fit properly in the culture plates. This procedure, however, was found to be too awkward, and the following step-by-step plan was therefore developed for all subsequent components.

After each component had been asceptically removed from its container and placed on a sterile tray, sterile shears were used to cut the thermocouple wire at its junction with the heat shield material, and the wire was placed in a peptone water bottle. Using two pairs of sterile pliers (one to hold the skin and its adherent heat shield material) the four brackets were pulled from the skin and all four were placed in a single peptone water bottle. Finally, the skin and its adherent heat shield material were sheared lengthwise into approximately equal parts. With component #SN-36 each of the two halves was immediately placed in its individual peptone water bottle, but to insure proper immersion in the peptone water subsequent component half sections were cut into quarters and the two quarter sections placed in a single bottle. As a control for each component, a sterile stainless steel strip was also placed in a peptone bottle. The component parts and the sterile stainless steel control strips were then bio-assayed according to the test plan. The assay results are presented in Tables II-VIII A through II-VIII E. The overall averages of colonies per base assembly are: non-heat shocked aerobic, 7.4 per assembly, anaerobic, 11 per assembly; and heat shoked aerobic, 4 per assembly; and anaerobic, zero. Each base assembly has a total surface area of approximately 35 square inches.

TABLE II- VIII A

BIO-ASSAY RESULTS ON BASE ASSEMBLIES

Base Assembly SN-#45

Parts: Tube #1 - bracket and skin and heat shield material
Tube #2 - bracket and skin and heat shield material
Tube #3 - bracket and skin and heat shield material
Tube #4 - thermocouple wire
Tube #5 - control plate

Anaerobic Culture Controls

A. faecalis - all negative
C. sporogenes - all positive

Tube #	Non-Heat Shocked Rinse Aliquot		Plated Parts	Heat Shocked Rinse Aliquot	
	Aerobic	Anaerobic	Aerobic	Aerobic	Anaerobic
1	1 0	0 0	0	0 0	0 0
2	0 0	0 0	0	1 0	0 0
3	0 2	0 0	0	1 0	0 0
4	1 0	0 0	3	0 0	0 0
5	0 0	0 0	0	0 0	0 0
Note 1 Total counts per component (non-heat shocked aerobic includes plated parts)	23	0		10	0

NOTE 1: Calculation of total counts:

Aliquot fraction per tube = 1/5

Total count = $\sum \frac{\text{culture count}}{\text{Aliquot fraction}}$

For aerobic non-heat shocked, the culture count from the plated parts is added to the summation to give the total count.

TABLE II-VIII B

BIO-ASSAY RESULTS ON BASE ASSEMBLIES

Base Assembly SN-#36

Parts: Tube #1 - brackets (4)
Tube #2 - skin & heat shield (One 1/2 section)
Tube #3 - skin & heat shield (One 1/2 section)
Tube #4 - thermocouple wire
Tube #5 - control plate

Anaerobic Culture Controls

A. faecalis - all negative
C. sporogenes - all positive

Tube #	Non-Heat Shocked Rinse Aliquot		Plated Parts	Heat Shocked Rinse Aliquot	
	Aerobic	Anaerobic	Aerobic	Aerobic	Anaerobic
1	0 0	0 0	0	0 0	0 0
2	0 0	0 3	0	1 0	0 0
3	0 0	0 0	0	0 0	0 0
4	0 0	0 0	0	0 0	0 0
5	0 0	0 0	0	0 0	0 0
Note 1 Total counts per component (non-heat shocked aerobic includes plated parts)	0	15		5	0

NOTE 1: Same as Note 1 on Table II-5A

TABLE II-VIII C

BIO-ASSAY RESULTS ON BASE ASSEMBLIES

Base Assembly SN-#42

Parts: Tube #1 - brackets (4)
Tube #2 - skin & heat shield (Two 1/4 sections)
Tube #3 - skin & heat shield (Two 1/4 sections)
Tube #4 - thermocouple wire
Tube #5 - stainless steel control strip

Anaerobic Culture Controls

A. faecalis - all negative
C. sporogenes - all positive

NOTE: Peptone volume was 100 cc for this component.

Tube #	Non-Heat Shocked Rinse Aliquot		Plated Parts	Heat Shocked Rinse Aliquot	
	Aerobic	Anaerobic	Aerobic	Aerobic	Anaerobic
1	0 0	4 0	0	0 0	0 0
2	Invalid - Sample dropped				
3	0 0	0 0	0	0 0	0 0
4	0 0	0 0	4	0 0	0 0
5	0 0	0 0	0	0 0	0 0
Note 1 Total counts per component (non-heat shocked aerobic includes plated parts)	4	40		0	0

NOTE 1: Same as Note 1 on Table II-5A except aliquot fraction per tube is 1/10.

TABLE II-VIII D

BIO-ASSAY RESULTS ON BASE ASSEMBLIES

Base Assembly SN-#21

Parts: Tube #1 - brackets (4)
Tube #2 - skin and heat shield (Two 1/4 sections)
Tube #3 - skin and heat shield (Two 1/4 sections)
Tube #4 - thermocouple wire
Tube #5 - stainless steel control strip

Anaerobic Culture Controls

A. faecalis - all negative
C. sporogenes - all positive

Tube #	Non-Heat Shocked Rinse Aliquot Aerobic	Non-Heat Shocked Rinse Aliquot Anaerobic	Plated Parts Aerobic	Heat Shocked Rinse Aliquot Aerobic	Heat Shocked Rinse Aliquot Anaerobic
1	0 0	0 0	0	0 0	0 0
2	0 0	0 0	0	0 0	0 0
3	0 2	0 0	0	0 1	0 0
4	0 0	0 0	0	0 0	0 0
5	0 0	0 0	0	0 0	0 0
Note 1 Total counts/ components (non-heat shocked aerobic includes plated parts)	10	0		5	0

NOTE 1: Same as Note 1 on Table II-5A.

TABLE II-VIII E

BIO-ASSAY RESULTS ON BASE ASSEMBLIES

Base Assembly SN-#44

Parts: Tube #1 - brackets (4)
Tube #2 - skin and heat shield (two 1/4 sections)
Tube #3 - skin and heat shield (two 1/4 sections)
Tube #4 - thermocouple wire
Tube #5 - stainless steel control strip

Anaerobic Culture Controls

A. faecalis - all negative
C. sporogenes - all positive

Tube #	Non-Heat Shocked Rinse Aliquot		Plated Parts	Heat Shocked Rinse Aliquot	
	Aerobic	Anaerobic	Aerobic	Aerobic	Anaerobic
1	0 0	0 0	0	0 0	0 0
2	0 0	0 0	0	0 0	0 0
3	0 0	0 0	0	0 0	0 0
4	0 0	0 0	0	0 0	0 0
5	0 0	0 0	0	0 0	0 0
Note 1 Total count/ component (non-heat shocked aerobic includes plated parts)	0	0		0	0

NOTE 1: Same as Note 1 on Table II-5A

b) Specimen Preparation for the Verification and Demonstration Cycles

(1) Preparation of fall-out strips for the bio-clean room in which the Assembly/Sterilizer Analog was installed.

Three hundred (300) stainless steel strips were prepared and cleaned as outlined below. (See Appendix B)

a) Wipe both sides with acetone
b) Wash with Haemo-Sol in hot water
c) Rinse three times with hot distilled water
d) Rinse with isopropanol
e) Rinse with ethyl ether
f) Dry

Just prior to use the strips were placed on stainless steel trays, in groups wrapped in aluminum foil, and sterilized.

(2) Preparation of Sterility Control Test Specimens

Five hundred seventy (570) stainless steel strips were prepared, cleared, and sterilized as above. Following sterilization, theu were inoculated with test organisms as indicated in the test plan (See Appendix B). Table II- IX identifies the organisms, and includes the calculated nominal populations, quantity of specimens prepared and counted, and the counted mean populations and standard deviations. Counting was done on a sampling basis only, as the bio-assay of the count on a specimen removes the population. Bio-assays were performed as outlined in the test plan (Appendix B).

A modification to the Test Plan was incorporated (See Appendix C) to include the use of ultrasonics to aid in the removal of spores from the sterility control strips. The spores could not be completely removed from the strips by immersing in peptone/tween diluent and shaking on a reciprocating shaker. All spores were completely removed after 5 minutes exposure to ultrasonics.

The maximum viable population of B. stearothermophilus which has been demonstrably achieved is 4×10^8. The spore suspension was prepared and harvested according to the test plan. When sporulation was microscopically 90 percent complete, direct counts on the number of spores was determined with a Petroff Hauser counting chamber. The results indicated a spore count of 5.5×10^{11}. However, when a viable count was made a concentration of 4.6×10^8 viable spores was found. This appears to be the highest concentration of spores which it is practical to prepare on a test specimen of this type, based on a viable count. Fifty stainless steel strips were prepared using the spore counts obtained in the Petroff Hauser chamber. The inoculum was 2.75×10^9 spores per strip. Four of these strips were bio-assayed with the results shown in group 6 of Table II-IX.

TABLE II-IX SUMMARY OF TEST SPECIMENS PREPARED

Group	Test Organism	Calculated Nominal Specimen Population	No. of Specimens		Counted Mean Populations and Standard Deviations	
			Innoculated	Counted	Heat Shocked	Non heat shocked
1	B. Subtilis var. niger	10^6	100	10	Mean: 1.2×10^5 Std. Dev. 0.28×10^5	Mean: 1.2×10^5 Std. Dev: 0.28×10^5
1A	B. subtilis var. niger	10^6	50	--	----------	----------
2	B. subtilis var. niger	10^8	100	10	Mean: 5.1×10^7 Std. Dev. 1.5×10^7	Mean: 6.0×10^7 Std. Dev. 0.96×10^7
2A	B. Subtilis var. niger	10^8	40	--	----------	----------
2B	B. Subtilis var. niger	10^8	30	--	----------	----------
3	B. Subtilis var. niger	10^9	100	10	Mean: 1.8×10^9 Std. Dev. 0.3×10^9	Mean: 2.1×10^9 Std. Dev. 0.35×10^9
4	B. Stearo-thermophilus	10^6	50	10	Mean: 2.4×10^5 Std. Dev. 0.39×10^5	Mean: 5.0×10^5 Std. Dev. 1.0×10^5
5	B. Stearo thermophilus	10^8	50	10	Mean: 1.1×10^8 Std. Dev. 0.22×10^8	Mean: 2.4×10^8 $\times 10^8$
6	B. Stearo thermophilus	10^9	50	4	Mean: 1.1×10^6 Std. Dev. 0.41×10^6	Mean: 2.75×10^6 Std. Dev. 0.87×10^6

The above table includes the additional sterility control specimens which were prepared due to two prematurely terminated verification cycles. Also included are the 40 specimens in the iron-constantan thermocouples attached. (Group 2A)

The highest calculated population of B. subtilus var niger spores that would consistently adhere to the sterility control specimens without excessive loss due to flaking off was approximately 2.5×10^9. The test plan (Appendix B) specified a nominal population of 10^{10}. The sterilization treatments prescribed by NASA Headquarters are predicated on a total lander population of 10^8. Thus, in every cycle, test specimens were used which had significantly greater populations than the populations on which the sterility treatments used in the program was based.

Part of the problem of higher assay counts may be due to the characteristic difficulty in heat activating spores of B. stearothermophilus. The counts obtained do not necessarily represent the true number of viable spores present, but represent only the number which were susceptible to the initial heat activation. This characteristic of the spores may be expected to vary from culture to culture, as those factors which control dormancy of B. stearothermophilus spores are not understood.

(3) Inoculation of One-half of the Structural Subassemblies to be used in the Demonstration Cycles.

Twenty-two (22) structural subassemblies were inoculated with nominal populations of 10^8 spores of B. subtilis var. niger. Inoculations were made on the aluminum skin near the point where the thermocouple lead-in wire was attached. No in situ counts were made, as bio-assays of these samples would have necessitated destruction of the subassemblies.

c) Test Results

(1) Verification Cycles

After installation and preliminary testing of the Assembly/Sterilizer Analog was completed, Verification Cycle #1 was initiated.

(a) Verification Cycle #1

Fifty sterility control test specimens were processed in the facility as indicated in Verification Cycle #1 in Appendix B. The test specimens were in four groups, (A, B, C, & D), each of which contained specimens with nominal populations of 10^6, 10^8, and 10^9 organisms. The utilization of these specimens is described in Table II-X.

TABLE II-X UTILIZATION OF STERILITY CONTROL SPECIMENS

Group	Quantity	Processing
A	10	ETO decontaminate specimens in auxiliary chamber
B	5	*ETO decontaminate container in auxiliary chamber
	10	*ETO decontaminate container in auxiliary chamber and sterilize specimens in main chamber
C	5	*ETO decontaminate container in auxiliary chamber
	10	*ETO decontaminate container in auxiliary chamber and sterilize specimens in auxiliary chamber
D	10	Sterilize specimens in autoclave

Group A, B, & C specimens seeded with B. subtillis var. niger;
Group D specimens seeded with B. stearothermophilus

*Specimens introduced into chamber in cans to verify that ETO/freon decontamination has little effect on populations unless in direct contact with them.

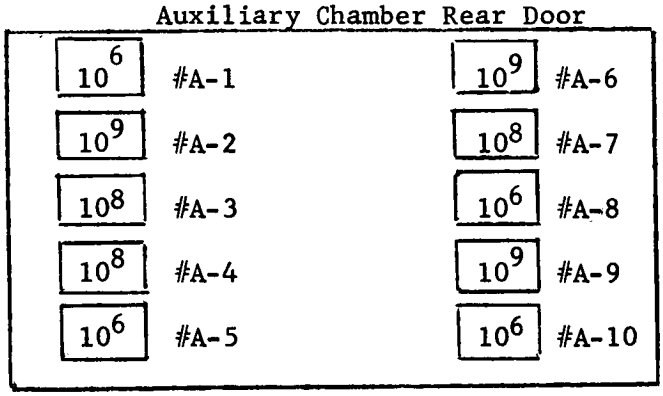
The fifty sterility control specimens were bio-assayed as follows:

- 10 specimens (Group A) to determine the effectivity of the ETO/FREON decontamination in the Auxiliary chamber.
- 5 specimens from each of two groups (B and C) to determine if any inadvertent decontamination of these specimens occured during their exposure, in sealed containers, to the ETO/FREON treatment in the auxiliary chamber.
- 10 specimens (Group C) to determine the efficacy of the dry heat sterilization in the auxiliary chamber.

- 10 specimens (Group B) to determine the efficacy of the dry heat sterilization in the main chamber.
- 10 specimens (Group D) to determine the efficacy of the wet heat sterilization in the autoclave.

Group A specimens were arranged in the auxiliary chamber as shown in Figure II-21. The numbers in the blocks indicate the nominal pretreatment populations.

FIGURE II-21 Group A Arrangement During ETO/FREON Treatment



The results of the Bio-Assay of Group A in Table II-XI were tabulated according to Standard Methods, American Public Health Association.

TABLE II-XI. GROUP A ASSAY RESULTS

Specimen	Population Pre-Treatment (nominal)	Post-Treatment
A-1	10^6	1.5×10^4
A-2	10^9	1.5×10^4
A-3	10^8	1.5×10^4
A-4	10^8	1.5×10^4
A-5	10^6	1.5×10^4
A-6	10^9	13
A-7	10^8	1.5×10^4
A-8	10^6	lost through breakage
A-9	10^9	0
A-10	10^6	2

(See next page for notes)

Note: (1) Plates were made with 1.0 ml and 0.1 ml aliquots of the 50 ml suspension of peptone/tween media. The remaining suspension including the stainless steel strips were then plated together.

Note: (2) A post ETO/FREON reexamination indicated that by plating the sterility control strip and peptone/tween suspension, colonies were too numerous to count. Therefore, the procedures for subsequent verification and demonstration cycles were modified (see Appendix C), permitting the remaining suspension to be plated in 5 ml aliquots.

Five specimens each from Group B and Group C as described in Table II-X were bio-assayed. The results are shown in Table II-XII.

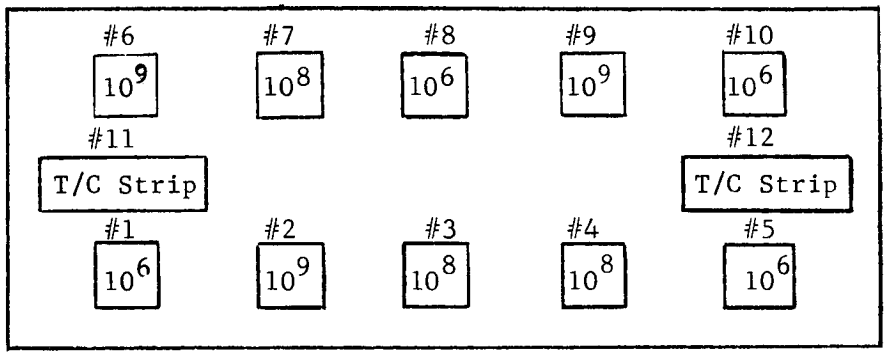
TABLE II-XII Bio-Assay of Group B and C Canned Specimens

Group	Specimen #	Population Pretreatment (nominal)	Post Treatment
B	11	10^6	6.0×10^4
	12	10^6	3.0×10^6
	13	10^8	1.9×10^7
	14	10^8	1.9×10^7
	15	10^9	7.0×10^8
C	16	10^6	1.5×10^4
	17	10^6	1.5×10^4
	18	10^8	1.5×10^6
	19	10^9	1.5×10^6
	20	10^9	1.5×10^7

The reduction of population of some of these specimens appears to have resulted from container seal leaks; steps were taken to prevent repetition of this problem, but subsequent data still showed some population reduction.

The specimens of group B exposed to the dry heat sterilization treatment were arranged in the main chamber as shown in Figure II-22. The assay of these specimens (post sterilization) showed no growth in a seven day incubation period.

FIGURE II-22 Group B Arrangement During
Dry Heat Sterilization



FRONT OF MAIN CHAMBER

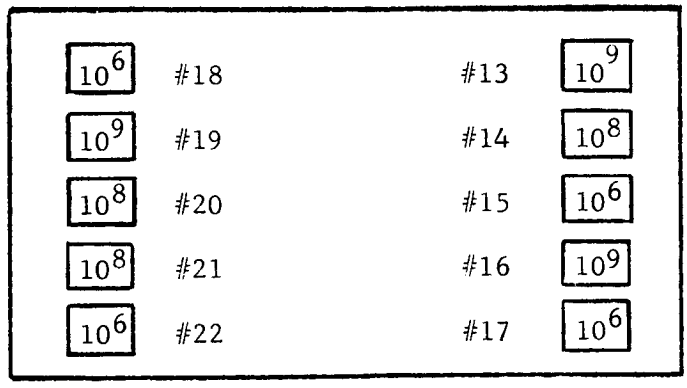
NOTE: 1. T/C strips had Iron Constantan thermocouples attached.

2. Numbers in blocks are nominal pre-treatment population.

The specimens of Group C exposed to the dry heat sterilization treatment were arranged as shown in figure II-23. The assay of these specimens (post sterilization) showed no growth in a seven day incubation period.

FIGURE II-23 Group C Arrangement During
Dry Heat Sterilization

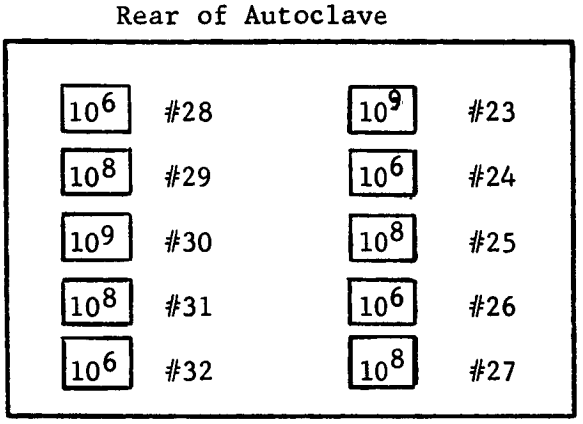
Rear of Auxiliary Chamber



NOTE: Numbers in blocks are nominal pre-treatment population

The specimens of group D were exposed to wet heat sterilization in the autoclave. They were arranged as shown in Figure II-24. The assay of the specimens (post sterilization) showed no growth in a seven day incubation period.

FIGURE II-24 Group D Arrangement During Wet Heat Sterilization



NOTE: The numbers in the blocks are nominal pre-treatment population

(b) Verification Cycle #2

Fifty sterility control test specimens were processed in the facility as in Verification Cycle #1. (See Table II-X). The test specimens were in four groups (A, B, C, & D) each of which contained specimens with nominal populations of 10^6, 10^8, and 10^9 organisms. The group A specimens were bio-assayed after ETO/FREON decontamination and yielded a spread of counts/strip from 3 to 71 with a mean of 15.1. The results of the individual specimens are presented in Table II-XIII.

TABLE II-XIII

BIO-ASSAY OF GROUP A SPECIMENS

Specimen #	Population Pre-Treatment (nominal)	Post Treatment
A-1	10^6	17
A-2	10^9	4
A-3	10^8	3
A-4	10^8	16
A-5	10^6	6
A-6	10^9	8
A-7	10^8	7
A-8	10^6	6
A-9	10^9	13
A-10	10^6	71

See Note 2 - Table II-XI

The Group B and C specimens represented nominal pre-treatment populations of 10^6, 10^8, and 10^9 organisms per strip. The assay of the 5 strips from each group which had had the containers exposed to ETO/FREON but had no further treatment yielded a spread of 7×10^3 to 1×10^9 counts/specimen. The data for the individual specimens is presented in table II-XIV.

TABLE II-XIV

Bio-Assay of Group B and C Canned Specimens

	Specimen #	Population Pre-Treatment (nominal)	Post-Treatment
Group B	11	10^6	3.3×10^4
	12	10^6	6.8×10^4
	13	10^8	2.4×10^7
	14	10^8	2.4×10^7
	15	10^9	1.0×10^9
Group C	16	10^6	1.9×10^5
	17	10^6	7.0×10^3
	18	10^8	8.9×10^6
	19	10^8	1.0×10^7
	20	10^9	8.9×10^7

The remaining group B and C sterility control strips were bio-assayed after dry heat sterilization and were found to be sterile. The thermocouple strips were also assayed and found to be sterile.

The group D specimens represented nominal pre-treatment populations of 10^6 10^8, and 10^9 organisms per specimen. All strips were assayed after steam sterilization and found to be sterile.

(c) Third Verification Cycle

Fifty sterility control test specimens were processed in the facility as indicated in Verification Cycles 1 and 2. The test specimens were in four groups (A, B, C, and D), each of which contained specimens with nominal populations of 10^6, 10^8, and 10^9 organisms.

The Group A specimens were bio-assayed after ETO/FREON decontamination and yielded a spread of 6,500 to 130,000 with a mean of 26,050. The results of the individual specimens are presented in Table II-XV.

TABLE II-XV

Results of Bio-Assay of Group A Specimens

Specimen #	Population Pre-Treatment (Nominal)	Post Treatment
A-1	10^6	1.7×10^4
A-2	10^9	1.7×10^4
A-3	10^8	1.5×10^4
A-4	10^8	1.3×10^5
A-5	10^6	1.6×10^4
A-6	10^9	1.1×10^4
A-7	10^8	1.7×10^4
A-8	10^6	1.5×10^4
A-9	10^9	1.6×10^4
A-10	10^6	6.5×10^3

The assay of the 5 strips from each of groups B and C which had had their containers exposed to ETO/FREON but had no further treatment yielded a spread of less than 1.5×10^4 to less than 1.5×10^8 counts per specimen. The data for the individual specimen is presented in Table II-XVI.

TABLE II-XVI

Bio-Assay of Group B and C Canned Specimens

	Specimen #	Population Pre-Treatment (Nominal)	Post Treatment
Group B	11	10^6	1.5×10^4
	12	10^6	1.5×10^4
	13	10^8	1.5×10^6
	14	10^8	1.5×10^6
	15	10^9	1.5×10^7
Group C	16	10^6	1.5×10^4
	17	10^6	1.5×10^4
	18	10^8	1.5×10^7
	19	10^9	1.5×10^7
	20	10^9	1.5×10^8

The remaining Group B and C specimens were assayed after dry heat treatment and were found to be sterile

The group D specimens were assayed after steam sterilization and found to be sterile.

(2) Demonstration Cycles

(a) Demonstration Cycle #1

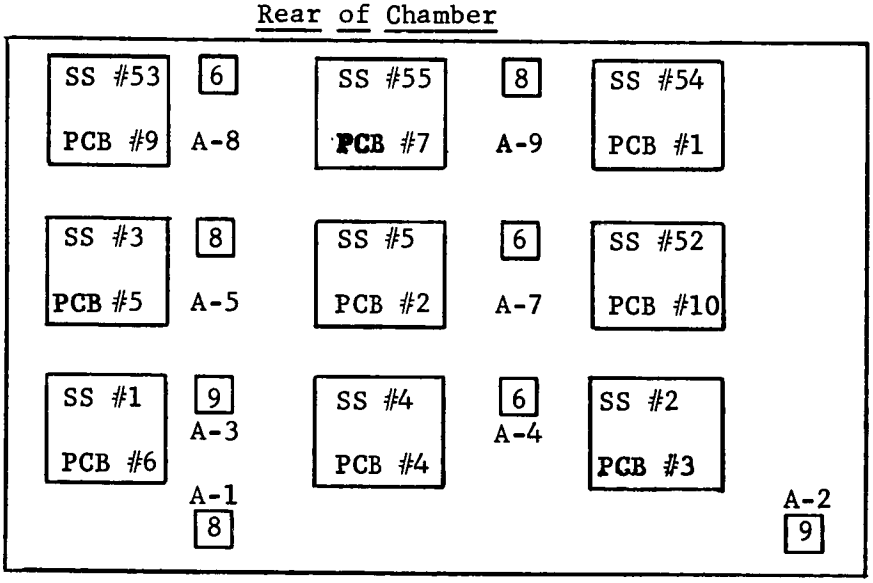
Nine test samples, 32 test specimens, and tools and test loads were processed in the facility as shown in Demonstration Cycle No. 1 in Appendix C. The test specimens were in three groups (A, B, and C), each of which contained specimens with nominal populations of 10^6, 10^8, and 10^9 organisms.

At the start of the test, the main chamber was decontaminated by dry heat. After decontamination of the main chamber, 9 test sample structural subassemblies and 9 printed circuit boards were placed in the auxiliary chamber with 9 sterility control specimens. Placement of these items in the chamber is illustrated in Figure II-25.

FIGURE II-25

PLACEMENT OF STERILITY CONTROL SPECIMENS
AND TEST SAMPLES IN AUXILIARY CHAMBER

DEMONSTRATION CYCLE NO. 1



NOTES; (1) Small rectangles represent S/C specimens and large rectangles represent test samples.

(2) Numbers in small rectangles represent $\log_{10}$ of the nominal population of the specimens.

(3) Number beside small rectangles give specimen identification.

(4) Numbers in large rectangles give serial numbers of structural subassemblies (SS#) and printed circuit Boards (PCB #).

(5) Structural subassemblies 1 through 5 were innoculated with 10^8 organisms of B. subtilis var. niger.

Assay of the group A specimens after ETO decontamination showed population reductions of 4 to 6 logs. The data for the individual specimens is presented in table II-XVII.

TABLE II-XVII

RESULTS OF BIO-ASSAY OF GROUP A SPECIMENS

DEMONSTRATION CYCLE NO. 1

SPECIMEN #	SPECIMEN POPULATION PRETREATMENT (Nominal)	POST-TREATMENT
A-1	10^8	200
A-2	10^9	350
A-3	10^9	20
A-4	10^6	10
A-5	10^8	5
A-6	10^9	7
A-7	10^6	6
A-8	10^6	25
A-9	10^8	40

In addition to the post-treatment counts shown in table II-XVII some of the plates contained colonies morphologically similar to A. niger. This organism is being used in large quantity in the bio laboratory in performance on contract NAS 1-6537 (BISS).

Prior to the ETO treatment, 18 sterility control specimens (Group B with the populations of 10^6 on 5 specimens, 10^8 on 7 specimens and 10^9 on 6 specimens) and 2 thermocouple strips (with 10^8 populations) were placed in the auxiliary chamber in a closed container. After the ETO treatment, they were transferred to the main chamber, removed from their container and placed on the main chamber grid. Nine were bio-assayed after dry heat sterilization. The remaining 9 plus the 2 thermocouple strips were assayed after the assembly and life test on the test samples. All specimens were found to be sterile.

After the life test, the test samples, with the exception of SS #55-PCB #7 were cut up and bio-assayed. All assayed samples were found to be sterile. (Because the assay procedure for test samples is destructive, one test sample was exempted from assay to permit accumulation of a small group of intact, processed demonstration test samples).

The 5 B. stearothermophilus control specimens of group C (With population of 10^6 for 2, 10^8 for 2, and 10^9 for 1) which were autoclaved with the final bio-assay material were all found to be sterile.

(b) DEMONSTRATION CYCLE NO. 2

Eight test samples, 23 test specimens, and tools and test leads were processed in the facility as shown in revised Demonstration Cycle No. 2 in Appendix C hereto.

The test specimens were in four groups (A, B, C, and D), each of which contained specimens with nominal populations of 10^6, 10^8, and 10^9 organisms.

At the start of the test, the main chamber was decontaminated by dry heat. After decontamination of the main chamber, 8 test sample structural subassemblies and 8 printed circuit boards were placed in the auxiliary chamber in conjunction with 8 sterility control specimens. Placement of these items in the chamber is illustrated in Figure LI-26.

FIGURE II-26

PLACEMENT OF STERILITY CONTROL SPECIMENS AND

TEST SAMPLES IN THE AUXILIARY CHAMBER

DEMONSTRATION CYCLE NO. 2

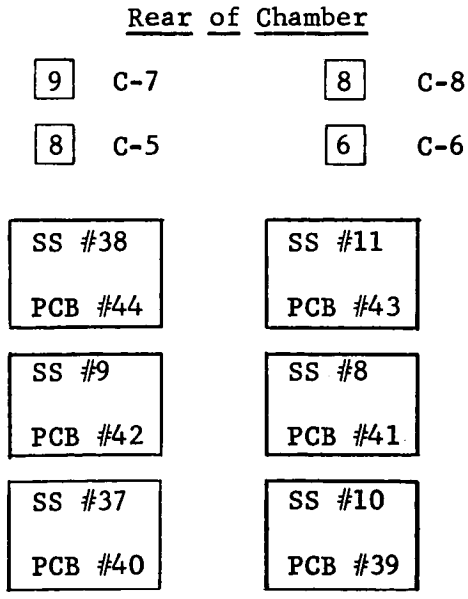
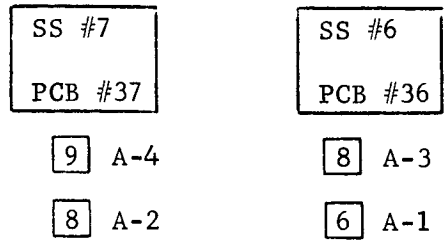


FIGURE II-26 (Cont'd)



NOTES: (1) Notes (1) through (4) of Figure II-25 apply here also.

(2) Structural subassemblies 6, 7, 9, and 10 were innoculated with 10^8 organisms of B. subtilis var. niger.

Assay of the group A specimens after ETO decontamination showed population reductions of 4 to 6 decades.

The data for the individual specimens is presented in Table II-XVIII.

TABLE II-XVIII

RESULTS OF BIO-ASSAY OF GROUP A SPECIMENS

DEMONSTRATION CYCLE NO. 2

SPECIMEN #	SPECIMEN POPULATION PRE-TREATMENT (Nominal)	SPECIMEN POPULATION POST-TREATMENT
A-1	10^6	15
A-2	10^8	140
A-3	10^8	88
A-4	10^9	186

Prior to the ETO treatment, 8 sterility control specimens (Group B with populations of 10^6 on 4 specimens, 10^8 on 2 specimens, and 10^9 on 2 specimens) and two thermocouple strips (with 10^8 populations) were placed in the auxiliary chamber in a closed container. After the ETO treatment, they were transferred to the main chamber, removed from their container, and placed on the main chamber grid. Four were assayed after dry heat sterilization along with the four specimens in Group C. The remaining four, plus the 2 thermocouple strips, were assayed after the assembly, simulated repair, and life test of the test samples. All specimens were found to be sterile.

After the life test, the eight test samples were cut up and bio-assayed. All were found to be sterile.

The 5 B. stearothermophilus control specimens of group D (with populations of 10^6 on 2, 10^8 on 2, and 10^9 on 1) which were autoclaved with the final bioassay material were all found to be sterile.

(c) DEMONSTRATION CYCLE NO. 3

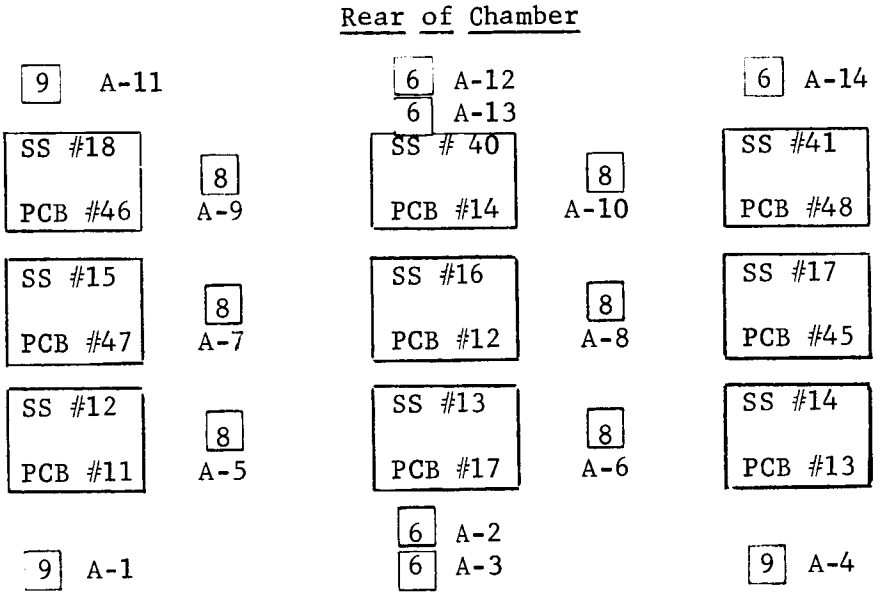
Fourteen test samples, 49 test specimens, five containers, and tools and test leads were processed in the facility as shown in Appendix C hereto. The test specimens were in three groups (A, B, and C) each of which contained specimens with nominal populations of 10^6, 10^8, and 10^9 organisms.

At the start of the test, the main chamber was decontaminated by dry heat. After decontamination of the main chamber, 14 test sample structural subassemblies and 14 printed circuit boards were placed in the auxiliary chamber with 14 sterility control specimens. Placement of these items in the chamber is illustrated in Figure II-27

FIGURE II-27

PLACEMENT OF STERILITY CONTROL SPECIMENS AND TEST SAMPLES IN THE AUXILIARY CHAMBER

DEMONSTRATION CYCLE NO. 3



Bottom Shelf

Rear of Chamber

SS # 49 PCB # 53	SS # 50 PCB # 54	
SS # 43 PCB #49	SS # 46 PCB # 50	SS # 47 PCB #52

NOTES: (1) Notes (1) through (4) of Figure II-25 apply here also.

(2) Structural subassemblies 12 through 18 and subassembly 40 were innoculated with 10^8 organisms of B. subtilis var. niger.

Assay of the group A specimens after ETO decontamination showed population reductions of 3 to 6decades. The data for the individual specimens is presented in Table II-XIX.

TABLE II-XIX

RESULTS OF BIO-ASSAY OF GROUP A SPECIMENS

DEMONSTRATION CYCLE NO. 3

SPECIMEN #	SPECIMEN POPULATION PRE-TREATMENT (Nominal)	SPECIMEN POPULATION POST-TREATMENT
A-1	10^9	< 150
A-2	10^6	< 210
A-3	10^6	< 30
A-4	10^9	< 30
A-5	10^8	< 100
A-6	10^8	< 100
A-7	10^8	< 100
A-8	10^8	< 30
A-9	10^8	< 300

SPECIMEN #	SPECIMEN POPULATION PRE-TREATMENT (Nominal)	POST-TREATMENT
A-10	10^8	< 300
A-11	10^9	< 300
A-12	10^6	< 300
A-13	10^6	< 300
A-14	10^6	< 300

Prior to the ETO treatment, 28 sterilty control specimens, (Group B with population of 10^6 on 10 specimens, 10^8 on 12 specimens, and 10^9 on 6 specimens) and 2 thermocouple strips (with 10^8 populations) were placed in the auxiliary chamber in a closed container. After the ETO treatment, they were transferred to the main chamber, removed from their container and placed on the main chamber grid. Fourteen of these were assayed after dry heat sterilization. The remaining 14 plus the 2 thermocouple strips were assayed after the assembly and life test of the test samples. All specimens were found to be sterile.

After the life test, 5 of the test samples were sealed in sterile containers to be recycled in Demonstration No. 4. These were:

(1) SS #13, PCB #17

(2) SS #14, PCB #13

(3) SS #15, PCB #47

(4) SS #43, PCB #49

(5) SS #46, PCB #50

The remaining test samples, with the exception of SS #50 - PCB #54 were cut up and bio-assayed. All assayed samples were found to be sterile. (Because the assay procedure for test samples is destructive, one test sample was exempted from assay to permit accumulation of a small group of intact, processed demonstration test samples).

The five B. stearothermophilus control specimens of Group C (with populations of 10^6 on 2, 10^8 on 2, and 10^9 on 1) which were autoclaved with the final bio-assay material were all found to be sterile.

(d) DEMONSTRATION CYCLE NO. 4

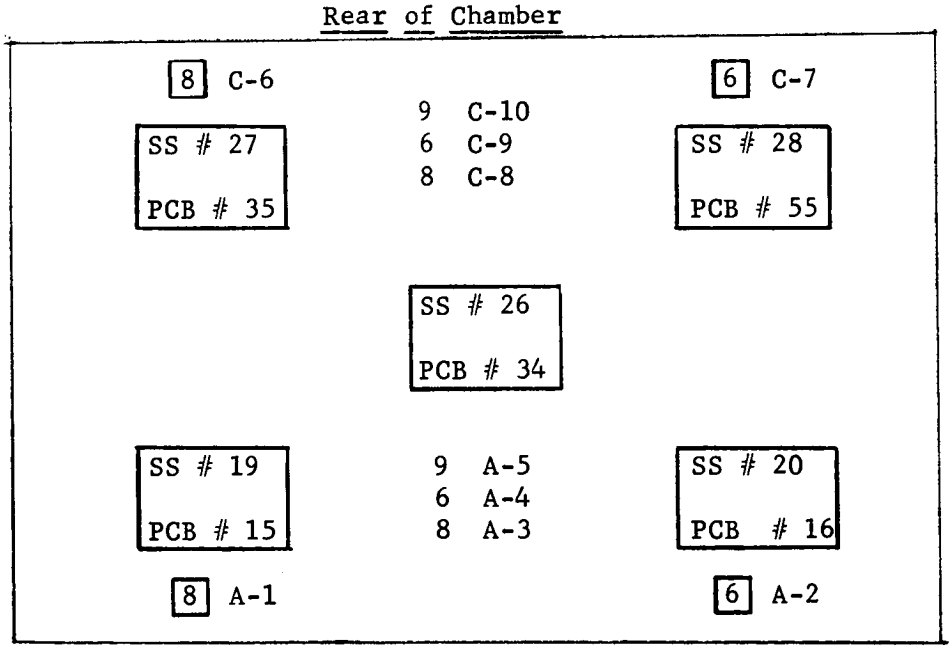
Ten test samples, 32 test specimens, and tools and test leads were processed in the facility as shown in the revised Demonstration Cycle No. 4 in Appendix C hereto. The test specimens were in five groups, (A, B, C, D, and E) each of which contained specimens with nominal populations of 10^6, 10^8, and 10^9 organisms.

At the start of the test, the test chamber was decontaminated by dry heat. After decontamination of the main chamber, 5 test sample structural subassemblies and 5 printed circuit boards were placed in the auxiliary chamber with ten sterility control specimens. Placement of these items in the chamber is illustrated in Figure II-28

FIGURE II-28

PLACEMENT OF STERILITY CONTROL SPECIMENS AND TEST SAMPLES IN THE AUXILIARY CHAMBER

DEMONSTRATION CYCLE NO. 4



NOTES: (1) Notes (1) through (4) of Figure II-25 apply here also.

(2) Structural subassemblies 19 and 26 were inoculated with 10^8 organisms of B. subtilis var. niger.

Assay of the group A specimens after ETO decontamination showed population reductions of 3 to 5 decades. The data for the individual specimens is presented in Table II-XX.

TABLE II-XX

RESULTS OF BIO-ASSAY OF GROUP A SPECIMENS

DEMONSTRATION CYCLE NO. 4

SPECIMEN #	SPECIMEN POPULATION PRE-TREATMENT (Nominal)	POST-TREATMENT
A-1	10^8	< 300
A-2	10^6	< 30
A-3	10^8	< 600
A-4	10^6	< 300
A-5	10^9	> 3000

Prior to the ETO cycle, 10 sterility control specimens (Group B with populations of 10^6 on 4 specimens, 10^8 on 4 specimens, and 10^9 on 2 specimens) and two thermocouple strips (with 10^8 populations) were placed in the auxiliary chamber in a closed container. After the ETO cycle, they were transferred to the main chamber, removed from their container and placed on the main chamber grid. Five were assayed after dry heat sterilization along with the five specimens in Group C. The remaining five specimens from Group B, the five from Group E and the 2 thermocouple strips were assayed after the assembly simulated repair, and life test of the test samples. All specimens were found to be sterile.

After sterilization of the five test samples in the main chamber, five samples from cycle No. 3 were introduced in sterile containers through the autoclave. The intent was to sterilize only the outside surfaces of the containers. Five B. stearothemophilus sterility control specimens (group D) were simultaneously introduced through the autoclave in similar containers as a control to assure that container contents were not sterilized during container outer surface sterilization.

After the life test, eight of the ten test samples were cut up and assayed (four from each of the two groups of samples processed). All were found to be sterile. (The remaining two samples, SS #19 - PCB #17, and SS #13 - PCB #15 were retained intact.

After all other material had been removed from the analog, the five specimens of group D were removed from their containers and assayed. This assay showed population reductions of less than two logs on 4 of the 5 specimens. Thus, the sterility indicated by the assay of the recycled test samples is properly interpreted as indicative of maintaining sterility on these samples during recycling, which is the desired result. The data from the individual specimens of Group D is presented in Table II-XXI.

TABLE II-XXI

RESULTS OF BIO-ASSAY OF GROUP D SPECIMENS

DEMONSTRATION CYCLE No. 4

SPECIMEN #	PRE-TREATMENT	POST-TREATMENT POPULATIONS	
		NON-HEAT SHOCKED	HEAT SHOCKED
D-1	10^6	1.3×10^4	1.1×10^4
D-2	10^6	3.7×10^4	3.7×10^4
D-3	10^8	2.0×10^6	1.7×10^4
D-4	10^8	4.9×10^4	4.4×10^6
D-5	10^9	$< 5 \times 10^5$	$< 5 \times 10^5$

(e) DEMONSTRATION CYCLE NO. 5

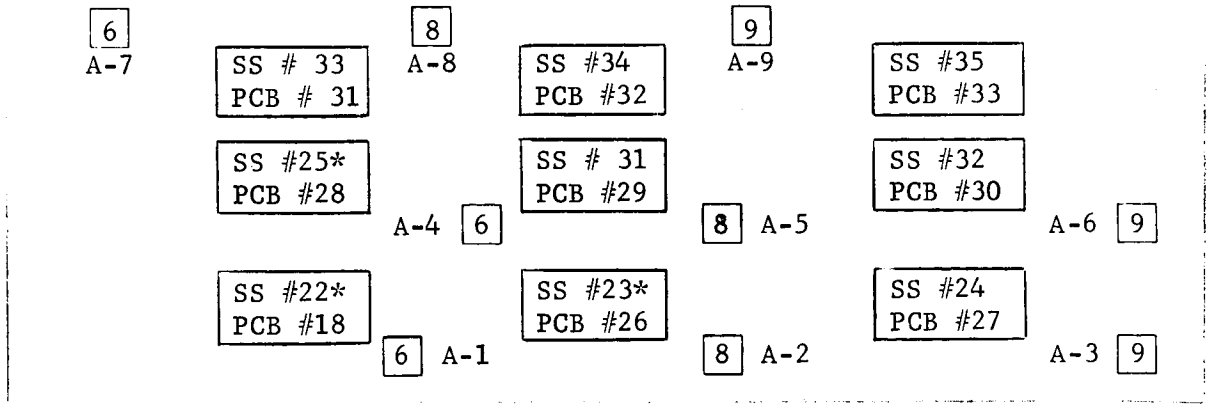
Nine test samples, 32 test specimens, and tools and test leads were processed in the facility as shown in the revised Demonstration Cycle No. 5 in Appendix C hereto (this is the same as cycle No. 1). The specimens were in three groups (A, B, and C) each of which contained specimens with nominal populations of 10^6, 10^8, and 10^9 organisms.

At the start of the test, the main chamber was decontaminated by dry heat. After decontamination of the main chamber, 9 test sample structural assemblies and 9 printed circuit boards were placed in the auxiliary chamber with 9 sterility control specimens. Placement of these items in the chamber is illustrated in Figure II-29.

FIGURE II-29

PLACEMENT OF STERILITY CONTROL SPECIMENS AND TEST SAMPLES IN THE AUXILIARY CHAMBER

DEMONSTRATION CYCLE NO. 5



Front of Chamber

NOTES: (1) Notes (1) through (4) of Figure II-25 apply here also.

(2) Structural subassemblies 22, 23, and 25 were innoculated with 10^8 organisms of B. subtilis var. niger.

Assay of the group A specimens after ETO decontamination showed population reductions of 3 to 5 decades. The data for the individual specimens are presented in table II-XXII.

TABLE II-XXII
RESULTS OF BIO-ASSAY OF GROUP A SPECIMENS

DEMONSTRATION CYCLE NO. 5

SPECIMEN #	SPECIMEN POPULATION PRE-TREATMENT (Nominal)	POST-TREATMENT
A-1	10^6	< 1500
A-2	10^8	< 1500
A-3	10^9	< 1500
A-4	10^6	< 1500
A-5	10^8	< 1500
A-6	10^9	< 1500
A-7	10^6	< 1500
A-8	10^8	< 1500
A-9	10^9	< 1500

Prior to the ETO cycle, 18 sterility control specimens (Group B with populations of 10^6, 10^8, and 10^9 on each of three subgroups of 6 specimens) and 2 thermocouple strips (with populations of 10^8) were placed in the auxiliary chamber in a closed container. After the ETO cycle, they were transferred to the main chamber, removed from their container, and placed on the main chamber grid. Nine were bio-assayed after dry heat sterilization. The remaining 9 plus the two thermocouple strips were assayed after the assembly and life test on the test samples. All specimens were found to be sterile.

After the life test, the test samples, with the exception of SS #31 - PCB #33 were cut up and assayed. All assayed samples were found to be sterile. (Because the assay procedure for test samples is destructive, one test sample was exempted from assay to permit accumulation of a small group of intact, processed demonstration test samples).

(f) AIR SAMPLING DURING DEMONSTRATION CYCLES

Air samples were taken in the horizontal laminar flow clean room during demonstration cycles 1 through 5 for a total of 48 samples. Twenty-four (24) samples were taken upstream of the analog, and twenty-four (24) samples were taken downstream of the analog. The results of this sampling are summarized in TableII-XXIII. The highest count from the 48 samples was 2.5 counts/ft^3.

TABLE II-XXIII

AIR SAMPLING RESULTS SUMMARY

POPULATION DENSITY RANGE (counts/ft^3)	FREQUENCY OF OCCURANCE Upstream	Downstream	Combined
.01 to .05	15	5	20
$.05^+$ to .10	4	5	9
$.10^+$ to .50	3	11	14
$.50^+$ to 1.0	1	3	4
Greater than 1.0	1	0	1
TOTAL	24	24	48

Average Population density for 24 Counts : Upstream .181 (counts/ft^3)
: Downstream .239 " "

Average Population density for 48 Counts : Combined .210 " "

The data for the individual air samples is presented in Table II-XXIV

TABLE II-XXIV

AIR SAMPLING BIO-ASSAY DATA FOR DEMONSTRATION

CYCLES # 1-5

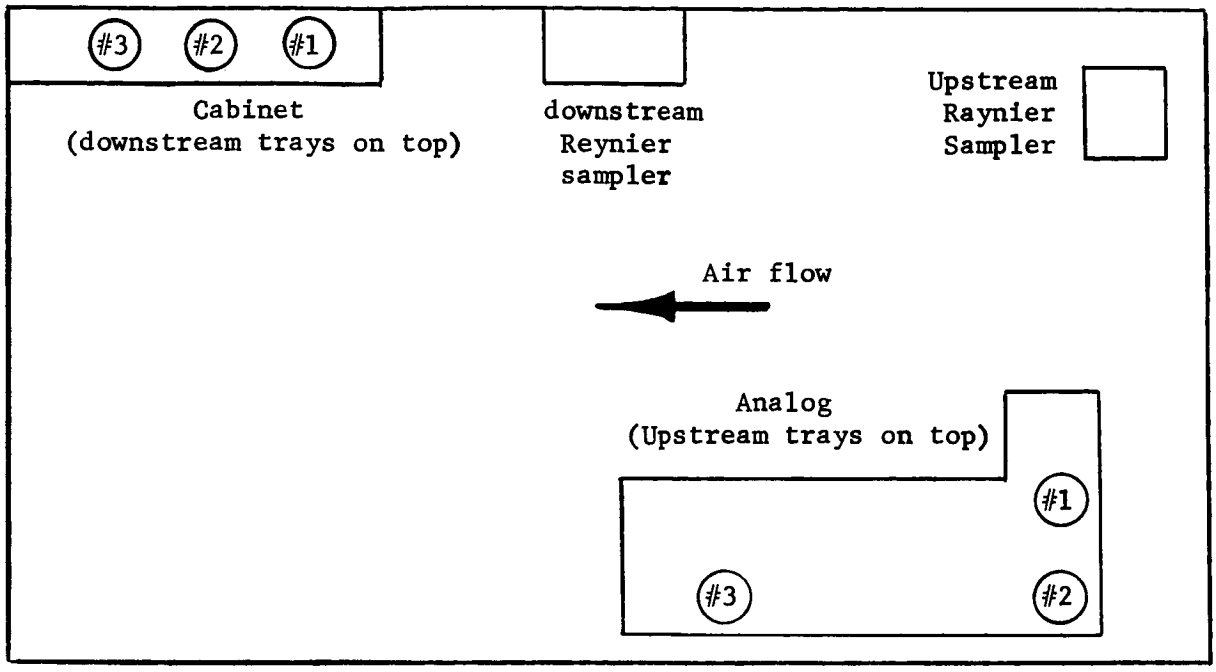
CYCLE #	TIME INTO CYCLE (0 time is at start of heat decontamination of main chamber)	ORGANISM DENSITY (Counts/ft^3) Upstream	Downstream
1	+ 20 hrs.	0.05	0.08
	+ 39 hrs.	0.03	0.1
	+ 46 hrs.	2.5	0.08

TABLE II-XXIV (Continued)

CYCLE #	TIME INTO CYCLE	POPULATION DENSITY	
		Upstream	Downstream
2	- 10 hrs.	0.03	0.37
	+ 15 hrs.	0.08	0.67
	+ 20 hrs.	0.03	0.3
	+ 39 hrs.	0.05	0.25
	+ 66 hrs.	0.1	0.45
3	- 6 hrs.	0.03	0.43
	+ 17 hrs.	0.17	0.58
	+ 69 hrs.	0.12	0.23
	+ 96 hrs.	0.03	0.07
4	- 4 hrs.	0.08	0.23
	+ 16 hrs.	0.02	0.13
	+ 33 hrs.	0.03	0.03
	+ 40 hrs.	0.02	0.03
	+ 69 hrs.	0.02	0.03
	+ 95 hrs.	0.02	0.13
5	- 137 hrs.	0.03	0.05
	- 87 hrs.	0.05	0.03
	+ 28 hrs.	0.65	0.75
	+ 55 hrs.	0.12	0.37
	+ 77 hrs.	0.06	0.25
	+ 100 hrs.	0.03	0.10

FIGURE II-30

PLACEMENT OF AIR SAMPLERS AND FALLOUT STRIPS
IN THE CLEAN ROOM



The samples were taken using Reynier samplers. One sampler was placed three feet above the floor at a point just upstream in the clean room from the analog, and another was placed three feet above the floor at a point near the downstream end of the room (See FigureII-30). The plates were incubated for 3 days and the number of viable counts per cubic foot of air was computed.

9. FALLOUT SAMPLING DURING DEMONSTRATION CYCLES

Six trays of one by two inch stainless steel fallout strips were placed in the laminar flow clean room as shown in figure II-30 (three trays upstream & 3 trays downstream). During cycle No. 1 one strip was removed from each tray and assayed. The remaining strips were sterilized and returned to the sampling sites where they remained throughout the rest of the test program.

Once per week during cycles 2 through 4, twice during the week preceeding cycle 5 and four times during cycle 5, one strip was removed from each tray and assayed. The maximum, minimum, and average counts/ft^3 were computed for the upstream and downstream sampling sites for each sampling time. The data from this sampling is presented in table II-XXV. These data are so widely spread that no pattern is evident. Larger sample quantities would have to be taken at much more frequent intervals to develop a pattern of accumulation and die off.

TABLE II-XXV

FALLOUT SAMPLING DURING DEMONSTRATION CYCLES

Cycle #	Hrs. into cycle	Days after strips were placed out	Counts/ft^2 UPSTREAM Aerobic NonH.S.	UPSTREAM Aerobic H.S.	UPSTREAM Anearobic Non H.S.	UPSTREAM Anearobic H.S.	SOWNSTREAM Aerobic Non H.S.	SOWNSTREAM Aerobic H.S.	SOWNSTREAM Anearobic Non H.S.	SOWNSTREAM Anearobic H.S.	
1	+37	1	9,000	4000	0	0	720	14,000	0		Maximum
			720	1100	0	0	430				Minimum
			4,000	2200	0	0	580				Average
2	+39	1	0	360	360	360	1400	360	720	0	Maximum
			0	0	0	0	720	0	0	0	Minimum
			0	120	120	120	1100	120	240	0	Average
3	+29	8	53,000	0	720	0	15,000	0	11,000	360	Maximum
			4,000	0	0	0	23,000	0	0	0	Minimum
			22,000	0	480	0	7,800	0	4,000	120	Average
4	+31	15	650	0	↑	↑	940	2200	↑	↑	Maximum
			0	0	spd	spd	360	360	spd	spd	Minimun
			360	0	↓	↓	430	840	↓	↓	Average
5	-144	34	360	0	720	19,000	21000	0	15,000	0	Maximum
			0	0	360	0	720	0	0	0	Minimum
			240	0	480	6,500	8200	0	5200	0	Average
5	-96	36	720	360	0	0	13000	360	3600	0	Maximum
			0	0	0	0	2200	0	360	0	Minimum
			360	120	0	0	7300	240	1600	0	Average
5	+29	41	14000	0	13000	0	1400	1100	7900	0	Maximum
			0	0	0	0	0	0	0	0	Minimum
			5200	0	4600	0	720	720	3400	0	Average
5	+56	42	1400	720	720	720	11000	1800	2200	4300	Maximum
			0	0	0	360	5200	0	720	0	Minimum
			600	240	360	600	7600	1200	1400	2000	Average

TABLE II-XXV (Cont'd)

Cycle #	Hrs. Into cycle	Days after strips were place out	Counts/ft^2 UPSTREAM Aerobic Non H.S.	UPSTREAM Aerobic H.S.	UPSTREAM Anearobic Non H.S.	UPSTREAM Anearobic H.S.	DOWNSTREAM Aerobic Non H.S.	DOWNSTREAM Aerobic H.s.	DOWNSTREAM Anerobic Non H.S.	DOWNSTREAM Anerobic H.S.	
5	+79	43	360	1100	360	3600	17000	1800	360	720	Maximum
		0	0	720	0	0	3000	360	0	0	Minimum
		44	120	9600	120	1300	8100	840	290	480	Average
5	+101	44	2200	360	5800	720	6100	360	2900	2200	Maximum
			0	0	360	0	2400	0	2200	720	Minimum
			740	120	2800	480	4300	120	2000	1300	Average

NOTE: The abbreviations in the tables are:

Non H.S. = Non heat shocked

H.S. = Heat Shocked

SPD = Spreader type colony (uncountable)

D. TASK 4. FULL SCALE FACILITY DESIGN STUDY

This task involved the performance of a preliminary design study on a full scale facility for processing of sterile flight vehicles using the Assembly/Sterilizer concept. The primary purpose of the study was to ascertain whether the design and construction of such a facility is feasible.

The complete study results are contained in Appendix E. This appendix summarizes the study, defines the facility concepts (with recommendation of the perferred concept), presents the results of the design analyses performed, and provides estimates of costs and schedules for the design and construction of the recommended facility.

The analysis portion in the study centered on the major design problem: a practical design for 400,000 cubic foot sealed vessel which operates at temperatures of 75^{o} F to 300^{o}F. A practical structual concept has been defined and the magnitude of the associated thermal design problem has been identified. More detailed study is needed in the thermal design area but was beyond the scope of the study.

The overall conclusion from the study is that the final design and construction appears technically and economically feasible. Though further studies are necessary to reinforce this conclusion and to provide a firmer basis for cost and schedule estimates, all investigations to date have had positive results.

III. ASSEMBLY/STERILIZER ANALOG

The Assembly/Sterilizer Analog provides a small scale simulation of the Assembly/Sterilizer. The Analog requirements were defined by G.E. and the equipment was designed and fabricated by Kewaunee Scientific Equipment.

The Assembly/Sterilizer Analog consists of three chambers - a main chamber, an auxiliary chamber, and an autoclave chamber. The main and auxiliary chambers are controlled dry sterilizers and the autoclave is a steam sterilizer. The main and auxiliary chambers are capable of using either nitrogen or air as the recirculating gas, and the auxiliary chamber is also capable of handling ethylene oxide/FREON-12 (ETO/FREON).

Primary sterilization is to be performed by recirculating dry heated sterile nitrogen, at less than 1% relative humidity within the main chamber, using the laminar flow principle and maintaining positive pressure within the chamber at all times. Although nitrogen is the gas intended for normal use, the A/S Analog is compatible with the use of other gases such as ethylene oxide, helium, and freon. (Although the present main chamber blower will not handle a gas of the density of ETO/FREON, a suitable blower is on hand and can be installed.)

In addition, the auxiliary chamber and autoclave are used to sterilize components which are introduced into the sterile main chamber after the primary sterilization cycle has been completed.

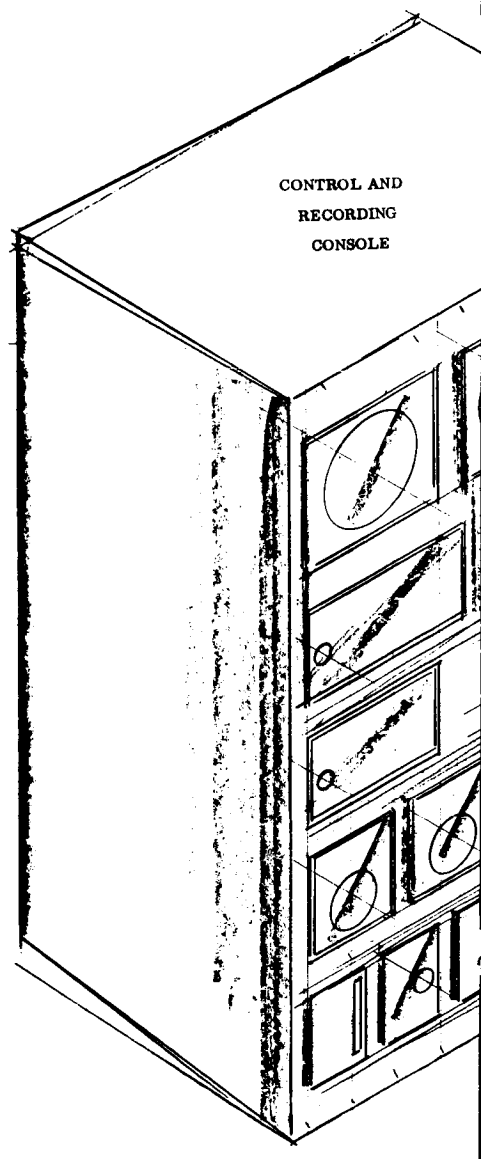
An artist's conception of the Assembly/Sterilizer Analog is shown in Figure III-I. This drawing shows the spatial interrelations of the several portions of the system. Photographs of the completed Analog are shown in Figures II-12 throughII-15.

The operating temperature of the main chamber is controllable from 70°F. to 300°F., and the operating temperature range of the auxiliary chamber is controllable from 100°F. to 300°F. The relative humidity in the chambers is controllable from 20% to 60% from 70°F. to 90°F, and at less than 1% from 200°F. to 300°F. When ETO is the recirculating gas, the temperature is controllable between 100°F. and 150°F. at a relative humidity of 50% to 60%. At no time will the relative humidity within either chamber exceed 60%. The temperature rate of change on heat-up or cool-down is 25°F. to 50°F. per hour for both chambers.

A flash heat exchanger to vaporize the Ethylene-Oxide Freon (ETO) used in the system is provided. A set of 650°F., 1.5 second, incinerators is also provided to sterilize the Nitrogen used in the system.

The main chamber is constructed of welded 304 stainless steel. The nominal inside working dimensions are 60" long x 24" deep x 30" high.

Access panels to the inside of the main chamber are located on both ends of the enclosure. The dimensions of the panels permit a 16" diameter disc to pass through the plane of the opening. These are bolt on service panels and are not used during routine operation of the Analog.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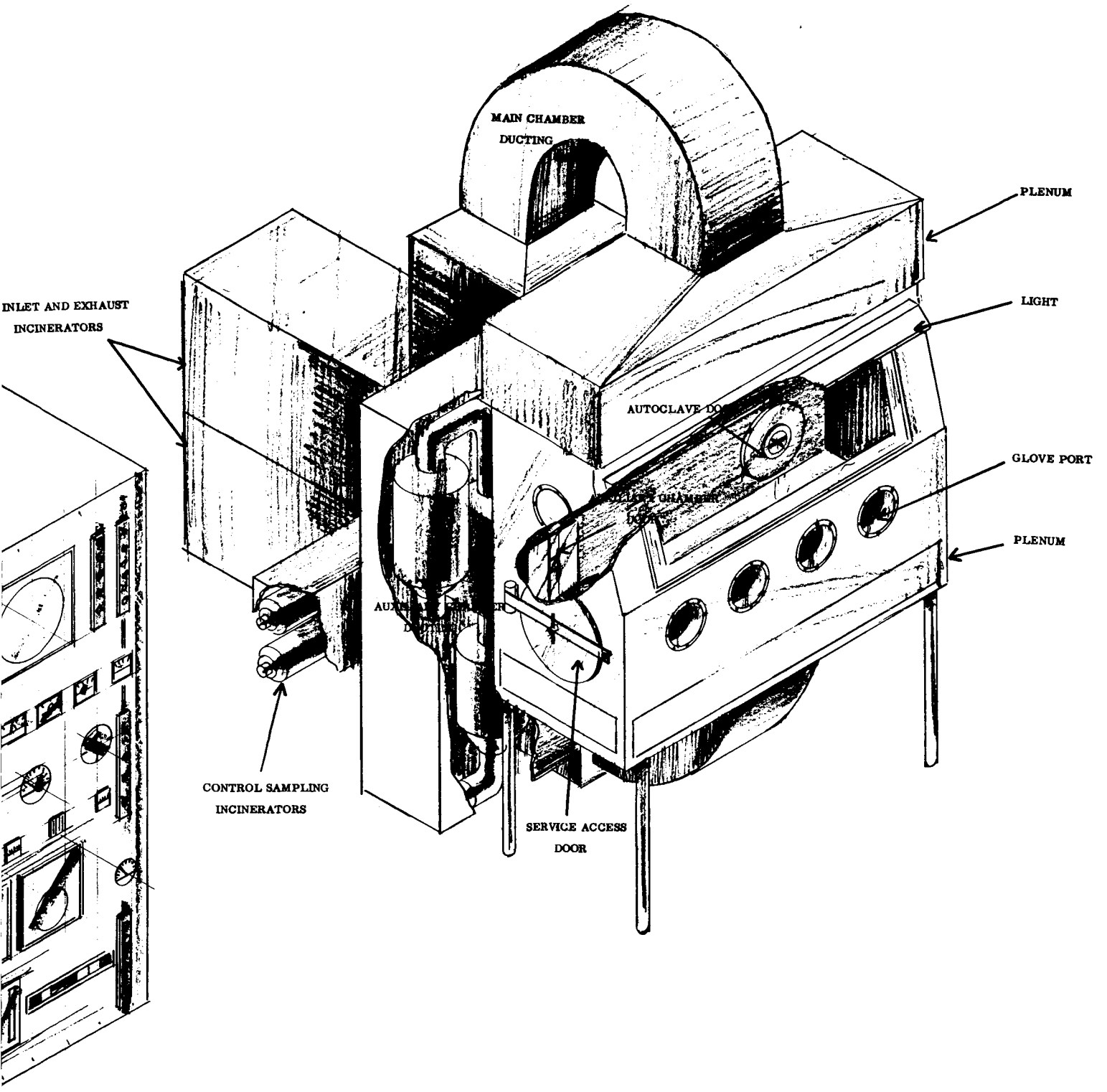

Figure III-I - Assembly/Sterilizer Analog

A large sloping observation window made from heat resistant, shatterproof, glass plate is provided on the front of the main chamber. To aid in the retention of heat during the sterilization cycle, a removable cover is used over the window. Two 8" ports, suitable for observation of the interior of the chamber, are incorporated in the cover.

Interconnected electrical junction panels with ten terminals each are located inside and outside the main chamber on the back surface. The terminals provide for banana plug and stripped wire connections between the inside and outside of the chamber.

Twenty permanent-type sheathed thermocouples are provided inside the chamber.

Two sets of gloves are provided on the front panel of the chamber. The gloves are made of 30 mil sulfur-free butyl rubber giving a glove which is soft, pliable, leakproof, and is capable of repeated exposure to all the environments specified for the equipment.

The chamber is capable of being purged with dry nitrogen until essentially all of the air is removed. (Approximately 20 volume changes per hour.) Flow rate meters are provided. Provision is incorporated for biological assay of the main chamber atmospheres.

The chamber is capable of continuous recirculation of the gas at a preselected velocity while maintaining the chamber in accordance with the environments specified. The makeup nitrogen gas is incinerated prior to entrance to the main chamber.

Two filters are provided in the design of the main chamber. One, a prefilter, is located in the floor of the chamber and the other, a final filter, is located in the top of the chamber. Both filters are removable for cleaning or replacement without disassembly of the chamber. The filters are such that the gas-borne particle count within the chamber will not exceed a total of 100 particles per cubic foot of 0.5 microns and larger. The floor of the main chamber is a grating with openings of one-half inch by one inch.

The auxiliary and autoclave chambers are attached to the back of the main chamber. The design of the doors between the auxiliary and autoclave chambers and the main chamber is such that the doors can be readily opened with a gloved hand. The doors open and slide in a horizontal plane.

The A/S Analog is so designed that the outside surface temperature of the chamber does not exceed 100°F. at any operating temperature and an ambient temperature of 70°F. to 80°F.

The main chamber maintains a controllable pressure gradient of up to 4" of water. The chamber does not exhibit any leak detectable with a helium mass spectrometer.

The auxiliary chamber design and construction is similar to the main chamber, except that the approximate size is 10" square x 16" long. The access door to the auxiliary chamber is located on the back of the chamber and permits complete uninhibited access to the chamber. Shelves are provided so that work pieces may be withdrawn. The shelves are capable of being withdrawn either into the main chamber from the front or out of the auxiliary chamber from the back.

Six sheathed thermocouples are provided and are of sufficient length to allow the thermocouples to be disconnected from the chamber contents with the gloved hand when the shelves are withdrawn into the main chamber. The chamber is capable of being purged with dry nitrogen until essentially all of the air is removed. (Approximately 20 volume changes per hour.) Flow rate meters are provided.

The auxiliary chamber is capable of recirculating the gas in a path independent of the main chamber but is not laminar flow. One filter is provided in the design of the auxiliary chamber and is capable of being removed for cleaning or replacement without disassembly of the chamber.

The outside surface of the auxiliary chamber does not exceed 100°F. at any operating temperature and an ambient temperature of 70°F. to 80°F. The auxiliary chamber maintains a controllable pressure gradient of up to 4" of water. The chamber does not exhibit any leak detectable with a helium mass spectrometer.

The autoclave chamber is a horizontal, vacuum pressure type vessel of approximately 18" diameter x 26" long, designed for operating pressures up to 30 psig. All details of design, materials and construction meet or exceed the requirement of the American Society of Mechanical Engineers code for unfired pressure vessels. The chamber is of welded 304 stainless steel.

The access door to the autoclave chamber is located on the back of the chamber and is of such a size as to permit uninhibited access to the chamber. The door is of the radial locking arm type. Shelves are provided inside the chamber to permit the work pieces to be withdrawn.

Six sheathed thermocouples are provided and are of sufficient length to allow the thermocouples to be disconnected from the autoclave contents with the gloved hand when the shelves are withdrawn into the main chamber.

The autoclave chamber is capable of being purged with dry nitrogen. (Approximately 20 colume changes per hour.) After the cooling cycle, and during purging, the autoclave will maintain a positive pressure relative to ambient.

The rate of steam admission is such that the autoclave chamber pressure can be raised to 30 psig in five minutes with an empty chamber.

The control console contains the instruments and circuitry for controlling the Analog. The following table identifies the instruments and their functions.

TABLE III-I. ANALOG CONTROL CONSOLE INSTRUMENTS (Note 1)

INSTRUMENT	FUNCTION
Temperature Controller/Recorder	1. Control and record main chamber gas temperatures. 2. Control and record auxiliary chamber gas temperatures.
Recorder 24 Channel Multipanel	Record skin temperatures and relative humidities of main and auxiliary chambers.
Meter/Relay Temperature Controllers (6)	1. Main chamber oven temperature. 2. Auxiliary chamber oven temperature. 3. Main chamber N_2 incinerator. 4. Auxiliary chamber N_2 incinarator. 5. ETO heat exchanger. 6. Autoclave N_2 incinerator.
Pressure Recorder (3 channel)	Record gas pressure in Analog chambers.
Differential Temperature Controllers (7)	Slave Analog skin temperatures to gas temperature.
RH Controllers	Control RH in main and auxiliary chambers.
Timers (3)	1. Time autoclave gas plumbing sterilization. 2. Relay main chamber incinerators while pumping ETO out of chamber. 3. Relay auximilary chamber incinerators while pumping ETO out of chamber.
Flow Meters (7)	Measure supply gas flows to Analog and RH sample gas flows.

Note 1. The autoclave steam cycle controls are located on the autoclave rather than on the console.

IV. RECOMMENDATIONS

The success of the present program suggests that the overall recommendation be that the Assembly/Sterilizer be considered as an economically and technically feasible facility for processing sterile interplanetary flight vehicles. It is recognized that feasibility has been more inferred than proven on the program. But viewing the weight of the results in light of the limited fiscal and work statement scope of the program, the inference is most strong.

Before any new facility of the complexity and sophistication of the Assembly/Sterilizer can be endorsed without qualification, investigation beyond the scope of the present contract studies is needed. The following recommended investigations are regarded as those which should remove any reservation as to the feasibility of the facility.

Perform a thermal analysis of the Assembly/Sterilizer facility in sufficient detail to size heat exchangers and wall modules, and design and analyze a thermal control system in sufficient depth to identify the types and numbers of all elements of the system.

Review the concept of the use of vertical laminar flow in the Assembly/Sterilizer to trade-off the improvement it offers in physical and biological cleanliness of the chamber environment before sealing-off against the complexity it adds to the structure, thermal control system, and gas movement system.

Investigate door and window openings to select optimum configurations. In particular, examine window and door thermal design problems, door seals, door supports, and door latching mechanisms.

Investigate bio-confidence monitoring systems based on physical measurements on the chambers or their contained gases. In particular investigate the use of gas leak detection techniques in terms of sensitivity, cost, and confidence enhancement.

Perform a gas management study to define the sizes and quantities of storage facilities, plumbing, and gas processing units. One area in particular that deserves further study is the design of an on-site facility for mixing and processing ethylene oxide and FREON-12 to achieve the desired concentrations in the chambers. Gas reclamation systems should be examined to permit an accurate gas economics analysis.

Design and construct a room size model of the Assembly/Sterilizer for testing the Bio-Isolator Suit System and the chamber control systems. Such a facility would verify by experimentation the validity of the design analyses on major portions of the full scale facility and would provide a facility for processing small flight vehicles.

In addition to the above recommendations directed at improving the confidence in assertions of feasibility of the facility, the following studies would enhance the contribution the Assembly/Sterilizer will make to interplanetary programs.

Perform a work flow analysis on the mast current Voyager design definition to optimize the facility for processign the flight hardware. This analysis is necessary to permit improved evaluation of the sizing of the chambers and support areas of the facility, the flow within the facility, and the operating modes of the facility chambers (eg. final decision on use of ETO/FREON in Assembly/Sterilizer main chamber.)

Investigate the long range interplanetary programs projections to determine the applicability of the Assembly/Sterilizer to program after Voyager.

Finally, it is recommended that the Assembly/Sterilizer Analog be seriously considered in planning and experimental efforts directed at the determination of the total physical and biological effects of dry heat sterilization of candidate materials, parts, components, and small assemblies for sterile flight vehicles. The analog is the only existant facility which permits physical and electrical access to decontaminated and sterilized equipment without the access provisions altering the biological environment; which permits true sterile insertion; and which permits performance of biological assays under sterile conditions.